Photosynthetic pigments and model compounds studied by pulse radiolysis

Jensen, N.-H.

Publication date:
1980

Document Version
Publisher's PDF, also known as Version of record

RISØ

Risø-R-415

Photosynthetic Pigments and Model Compounds Studied by Pulse Radiolysis

Niels-Henrik Jensen

Risø National Laboratory, DK-4000 Roskilde, Denmark
May 1980

Risø-R-415

PHOTOSYNTHETIC PIGMENTS AND MODEL COMPOUNDS STUDIED BY PULSE RADIOLYSIS

Niels-Henrik Jensen
Chemistry Department

Abstract. The photosynthetic pigments chlorophyll a and all-trans-β-carotene as well as the quinone model compound duroquinone have been studied in solution by pulse radiolysis combined with time-resolved absorption and resonance Raman spectroscopy.

In benzene solution the excited triplet states of the substrates were produced either directly in the case of duroquinone or by triplet energy transfer from triplet naphthalene in the case of chlorophyll a and β-carotene.

All relevant rate constants involved in the reactions of the excited states in benzene were determined, including

i) the rate constants for energy transfer from triplet naphthalene to chlorophyll a with $k = (3.6\pm0.6)\cdot10^9\ M^{-1}s^{-1}$ and β-carotene with $k = (10.7\pm1.2)\cdot10^9\ M^{-1}s^{-1}$

ii) the rate constants of triplet-triplet annihilation of
chlorophyll a: $(1.4\pm0.3)\cdot10^9\ M^{-1}s^{-1}$
β-carotene : $(3.6\pm0.4)\cdot10^9\ M^{-1}s^{-1}$
duroquinone : $(3.0\pm0.6)\cdot10^9\ M^{-1}s^{-1}$.

(Continued on next page)

May 1980
Risø National Laboratory, DK-4000 Roskilde, Denmark

For β-carotene it is suggested that triplet-triplet annihilation produces the optically forbidden excited ^1A_g state.

The first-order components of the triplet decays were strongly dependent upon irradiation dose in the case of naphthalene and duroquinone but apparently only slightly dependent on or independent of irradiation dose in the case of chlorophyll *a* and β-carotene. Apparent bimolecular rate constants for triplet quenching by radiolytically produced free radicals are determined. The triplet state of duroquinone is quenched by ground state duroquinone with a rate constant of $(1.2\pm0.3)\cdot10^6$ $M^{-1}s^{-1}$.

The excited triplet state of all-trans-β-carotene has been investigated by time-resolved resonance Raman spectroscopy. Six transient Raman bands at 965 cm^{-1}, 1009 cm^{-1}, 1125 cm^{-1}, 1188 cm^{-1}, 1236 cm^{-1} and 1496 cm^{-1} were observed. The spectra suggest that the C = C band order is decreased and that the molecule may be substantially twisted, presumably at the $15,15^1$ bond, in the triplet state.

The radical anion of chlorophyll *a* with λ_{max} = 455 nm has been produced in tetrahydrofuran (THF) and 2-propanol by reaction between solvated electrons and chlorophyll *a* with rate constants $k \geq 2\cdot10^{11}$ $M^{-1}s^{-1}$ in THF and $k = (1.5\pm0.2)\cdot10^{10}$ $M^{-1}s^{-1}$ in 2-propanol.

INIS Descriptors: ABSORPTION SPECTROSCOPY, BENZOQUINONES, CAROTENOIDS, CHLOROPHYLL, DOSE-RESPONSE RELATIONSHIPS, ENERGY TRANSFER, EXCITED STATES, NAPHTHALENE, PULSED IRRADIATION, RADICALS, RADIOLYSIS, RAMAN SPECTRA, REACTION KINETICS, TIME RESOLUTION, TRIPLETS.

UDC 547.979.7:541.15

ISBN 87-550-0710-4
ISSN 0106-2840
Risø Report 1980

CONTENTS

I ADMIT IT'S GETTING BETTER ....
WITH A LITTLE HELP FROM MY FRIENDS ....

(Lennon and McCartney, 1967)

FOREWORD

This report has been submitted to the University of Copenhagen in partial fulfilment of the requirements for obtaining the degree lic.scient. (Ph.D.). The work upon which the report is based was conducted at the Chemistry Department, Risø National Laboratory during the period 1976-1979, with Palle Pagsberg (Risø) and Niels Harrit (University of Copenhagen) as advisers. The work was financially supported by the Danish Natural Science Research Council and Risø National Laboratory.

The author is also grateful to Kjeld Engvild (Risø) who originally proposed the theme of the project and whose help and advice in the first phases was invaluable. Most of the expensive chlorophyll a used in this work was carefully prepared by Inga Nordstrøm (University of Copenhagen).

Likewise, the author is grateful to the staff of the Chemistry Department, especially to the members of the Radical Chemistry Group, for the four fruitful years.

Finally, the crucial role of my three lovely girlfriends Gunna, Maria, and Cecilia, who shared the ups and downs during this work, should be stressed.

Most of the ups and downs can be ascribed to the work involving time-resolved resonance Raman spectroscopy. The original idea of using resonance Raman spectroscopy in conjunction with pulse radiolysis is solely due to Palle Pagsberg and the first demonstrations of the feasibility of this powerful technique were also made at Risø (Wilbrandt et al., 1975; Pagsberg et al., 1976).

Later most of the efforts of the group have been concentrated on improving the technique and demonstrating its potential in the study of short-lived radicals and excited states. This work

have been published elsewhere:

1) R. Wilbrandt, N.H. Jensen, P. Pagsberg, A.H. Sillesen and K.B. Hansen
Triplet-state resonance Raman spectroscopy.
Nature (London) 276, 167-168 (1978).

2) R. Wilbrandt, N.H. Jensen, P. Pagsberg, A.H. Sillesen, K.B. Hansen and R.E. Hester
Resonance Raman spectrum of the transient $(SCN)_2^-$ free radical anion.
Chem. Phys. Lett. 60, 315 (1979).

3) N.H. Jensen, R. Wilbrandt, P. Pagsberg, R.E. Hester and E. Ernstbrunner
Vibrational analysis of $(SCN)_2$ and the transient $(SCN)_2^-$.
J. Chem. Phys. 71, 3326 (1979).

4) R. Wilbrandt, N.H. Jensen, P. Pagsberg, A.H. Sillesen, K.B. Hansen and R.E. Hester
Resonance Raman spectrum of the transient dimethylsulfide dimer radical cation, $[(CH_3)_2S]_2^+$.
Submitted to J. Raman Spectrosc.

1. INTRODUCTION

Photosynthesis, i.e. the process by which chlorophyll-containing organisms convert light energy to chemical energy (carbohydrates, ATP and O_2), is the most important biological process as it provides energy, food, and oxygen for all heterotrophic organisms. Photosynthesis of green plants is generally divided into the light and dark reactions. The dark reactions consume the final products of the light reactions, i.e. NADPH and ATP, and they are not directly dependent on the presence of light. The classical light reactions which constitute the so-called Z-scheme are, on the other hand, a series of coupled photochemical and non-photochemical electron transfer reactions, as depicted in Fig. 1. As seen in Fig. 1 photosynthesis of green plants is driven by two photochemical electron transfer reactions which take place at two distinct sites of the photosynthetic membrane. These sites of reaction (reaction centers) with their associated light-harvesting antenna systems are called Photosystem I and II. The light-harvesting chlorophyll-proteins (antenna) absorb the incoming light, and the excitation energy is rapidly and efficiently transferred to the reaction centers where the electronic excitation is transformed into a reducing radical anion and an oxidizing radical cation by electron transfer from the donors, P700 (in PSI) and P680 (in PSII), to the primary acceptors. P700 and P680 represent two different photoactive chlorophyll-protein complexes which have optical absorptions at 700 nm and 680 nm, respectively. The two photosystems are coupled by a series of electron transfer reactions involving plastoquinone, cytochrome *f* and plastocyanin. As all processes are one-electron processes it follows that transfer of one electron from H_2O to $NADP^+$ requires at least two photons, i.e. evolution of one molecule O_2 requires eight photons. In most cases the actual requirements are higher; the cyclic phosphorylation reaction from PSI to plastoquinone/cyt *f* can be considered as a short circuit although some energy is stored in the ATP produced. Thus, the term "light reactions" in this context means only that the reaction sequence of photosystems II and I is pumped by photon ab-

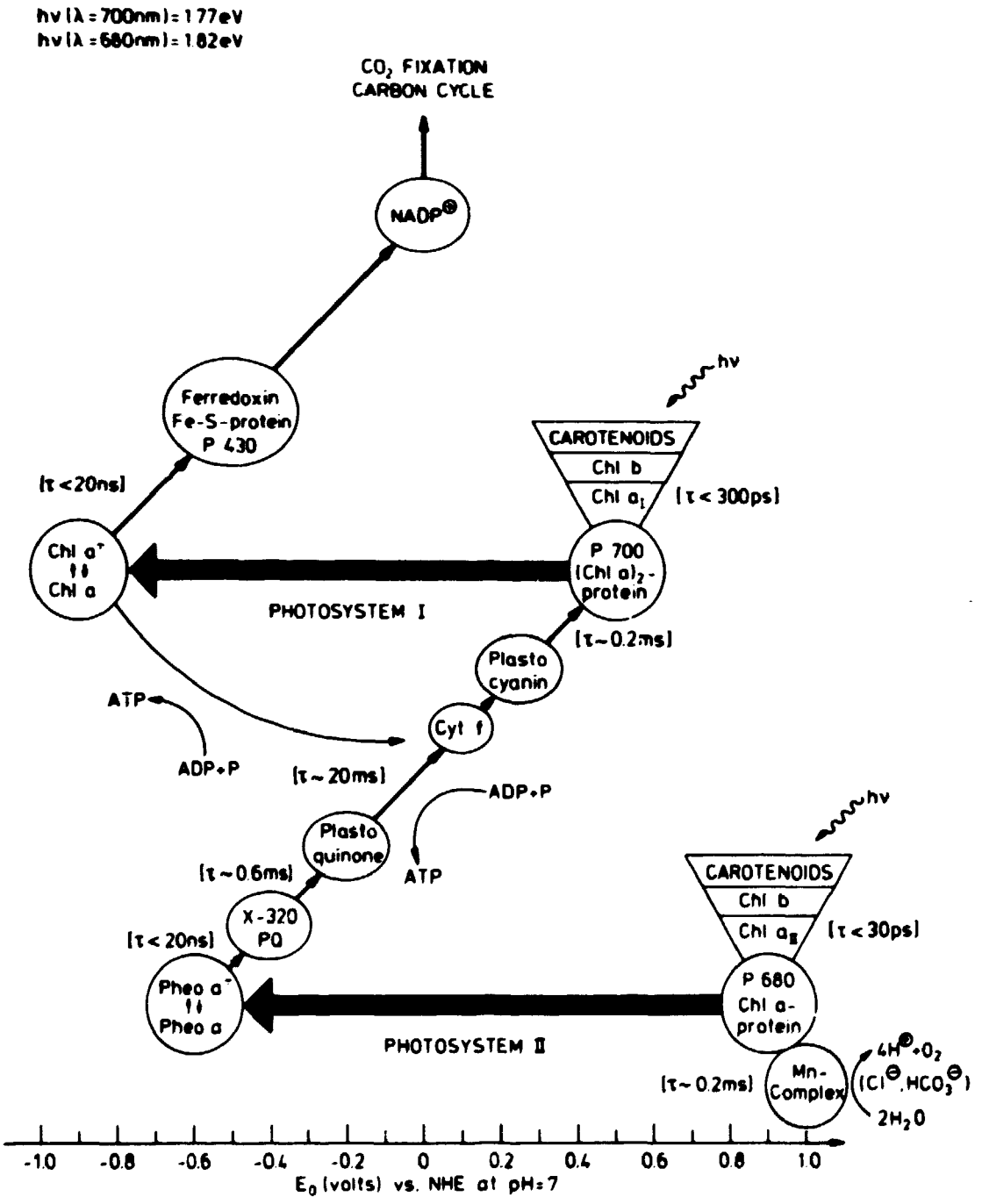


Figure 1. Updated Z-scheme of green plant photosynthesis.

sorption of the antenna systems of P 680 and P 700, but not that the individual reactions are photochemical. In fact, the photochemical/photophysical parts of the Z-scheme account for only ca. 500 ps of the ca. 20 ms, which is the typical time for transfer of one electron from H_2O to $NADP^+$ (Junge, 1977). In spite of this unequal distribution in time, the various photochemical aspects of the Z-scheme have been the ones to receive the most attention during the last 15 years (Vernon and Seely, 1966; Olson and Hind, 1977; Govindjee, 1975; Trebst and Auron, 1977; Sanadi and Vernon, 1978a; Porter, 1979).

Progress in our understanding of the photon-induced processes of photosynthesis has been extremely rapid during these years but still the picture is far from being satisfactory. Important problems, such as the nature of the primary charge separation and the chemical as well as structural factors making the charge separation effective, are still at the proposal stage. Even more obscure is the nature of the oxygen-evolving process in green plants, as virtually nothing is known about the chemical constitution of the probable manganese complex that participates in this crucial reaction, whereby water is oxidized to molecular oxygen under physiological conditions. Thus, photosynthesis still offers an immense number of unsolved structural, chemical, and physical problems.

This report describes studies of photosynthetic pigments by pulse radiolysis combined with time-resolved absorption and resonance Raman spectroscopy. The kinetic studies have been focused on reactions and conditions where the special advantages of pulse radiolysis compared with flash photolysis are utilized. In the field of time-resolved resonance Raman spectroscopy the experimentalist, roughly speaking, still has to be happy when something works. Thus, the degrees of freedom are limited when the systems to be studied are selected. Fortunately, one of the ideal candidates for study by time-resolved resonance Raman spectroscopy turned out to be the excited triplet state of ß-carotene, which is one of the most important photosynthetic pigments.

2. EXPERIMENTAL

2.1. Chemicals

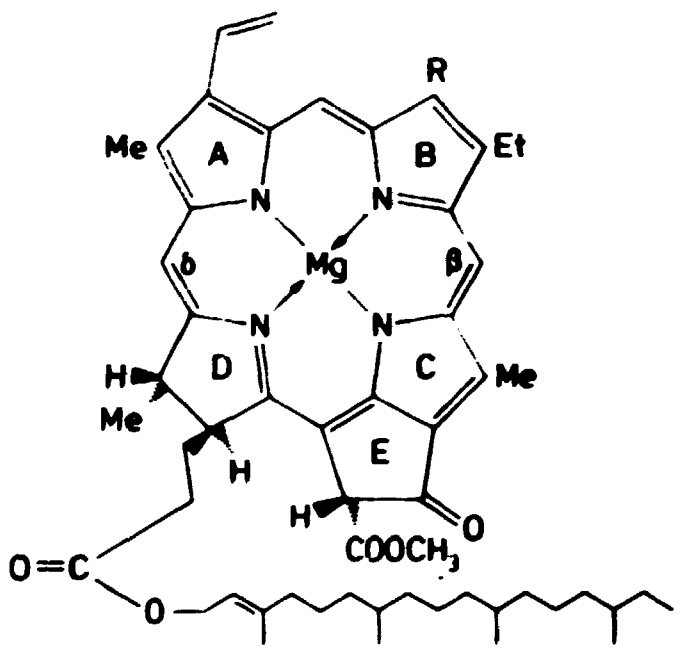
Chlorophyll a was prepared by a method which essentially is a combination of previously published procedures (Strain and Svec, 1966; Anderson and Calvin, 1962; Sauer et al., 1966), using chromatography of spinach extracts on icing sugar and polyethylene.

Fresh spinach leaves (ca. 200 g) were heated in boiling water for ca. 2 minutes. The leaves were cooled rapidly by pouring ice on them. After removing excess water from the leaves they were extracted with acetone (500-750 ml) for 1 hour. The leaves were removed from the deeply coloured extract by filtration. When necessary, the leaves were washed with additional 100-200 ml acetone. The combined extract (ca. 1000 ml) was divided into two portions. The extract was stored in the dark at -30°C until further purification.

Twenty per cent (v/v) water was added to the raw extract and the mixture (ca. 600 ml) was adsorbed by means of an ordinary pump on a chromatographic column dry-packed with polyethylene powder (Merck, for spectroscopy). The chlorophylls and carotenes are firmly adsorbed on this material while the xanthophylls are eluted. The column was washed with ca. 2000 ml 70% (v/v) aqueous acetone (5 ml/min) to completely remove the xanthophylls. The washing was continued until the eluate was colourless. Following this, the green chlorophyll fraction was eluted with 85% (v/v) aqueous acetone. At this stage the combined chlorophyll fraction was ca. 250 ml. The chlorophylls were transferred to 200-ml iso-octane by adding the iso-octane to the aqueous acetone fraction in a separatory funnel and washing the iso-octane phase with water until the chlorophylls began to crystallise from the iso-octane, indicating that the acetone was removed. Finally, the iso-octane was washed twice with saturated aqueous NaCl solution. The nearly black iso-octane phase contains crys-

tals of the chlorophyll hydrates at this stage. By adding 10-50 ml diethylether a homogeneous solution was obtained.

The final separation of the chlorophylls was made using a column dry packed with icing sugar. The iso-octane/diethylether solution was adsorbed and the column was eluted with iso-octane/0.5% 1-propanol. Only the middle of the chlorophyll a fraction was collected (50-100 ml) as a deeply coloured bluish solution. Chlorophyll a was isolated from this solution by washing the iso-octane fraction four times with 40% aqueous ethanol and ca. ten times with redistilled water. The washing was continued after the hydrate of chlorophyll a began to separate. The washed iso-octane phase was placed in the dark at -30° for several days after which chlorophyll a was isolated by centrifugation. The crystals (40-50 mg) were washed with cold n-pentane and dried in vacuum over solid KOH before storage in the dark at -30°C. The visible absorption spectrum in dry diethylether had A_{428}/A_{660} = 1.30±0.01, and reversed-phase HPLC showed only chlorophyll a and chlorophyll a' (Hynninen et al., 1979) in a sample stored for several months. Typical visible absorption spectra of chloro-



R = Me CHLOROPHYLL A
R = CHO CHLOROPHYLL B

phyll a prepared by this method are shown in Figs. 2 and 3. These spectra also illustrate the difference in solution of chlorophyll a in diethylether and tetrahydrofuran (Seely and Jensen, 1965; Shipman et al., 1976). In tetrahydrofuran the central Mg-atom of chlorophyll a is hexacoordinated while it is only pentacoordinated in diethylether.

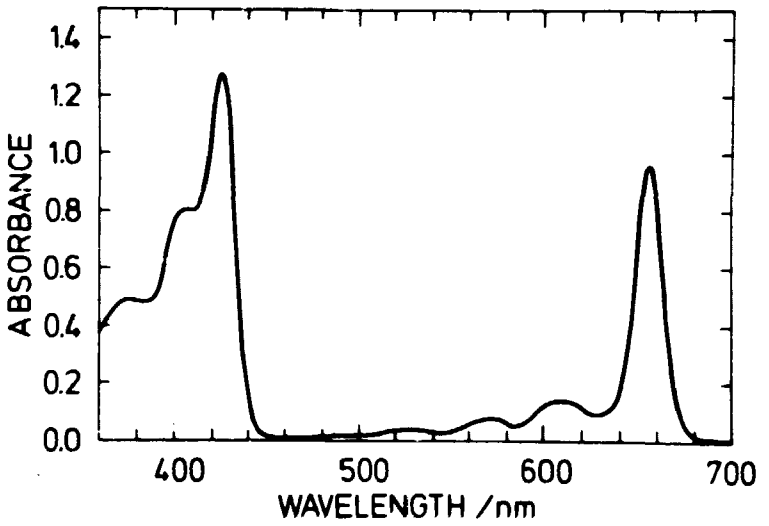


Figure 2. Visible absorption spectrum of chlorophyll a in diethylether.

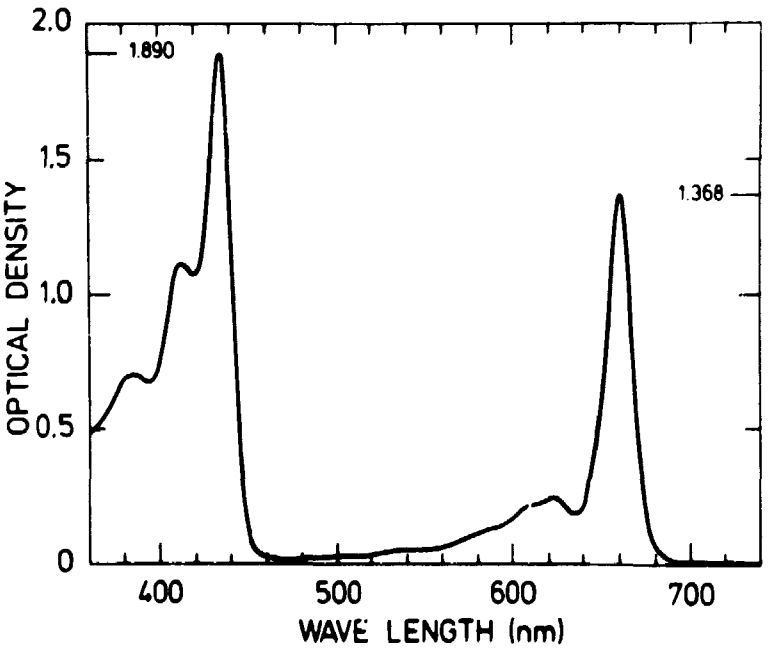
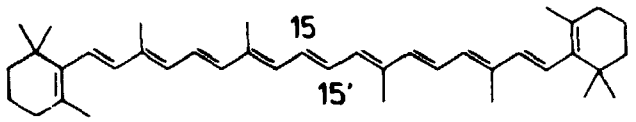


Figure 3. Visible absorption spectrum of chlorophyll a in tetrahydrofuran.

In some experiments (not included in the report) chlorophyll a obtained from Sigma was used.

All-trans-β-carotene was obtained from Sigma and used without further purification. Its absorption spectrum is shown in Fig. 4. Naphthalene was scintillation grade from Merck, and it was



ALL-trans β-CAROTENE

used as received. Duroquinone from Fluka was sublimated in vacuum one or two times before use. All solvents, including triethylamine, were analytical grade. They all were passed through a column packed with alumina (neutral or basic) before use. Tetrahydrofuran, however, was passed through basic alumina twice, in order to remove the stabilizer completely, and the purified

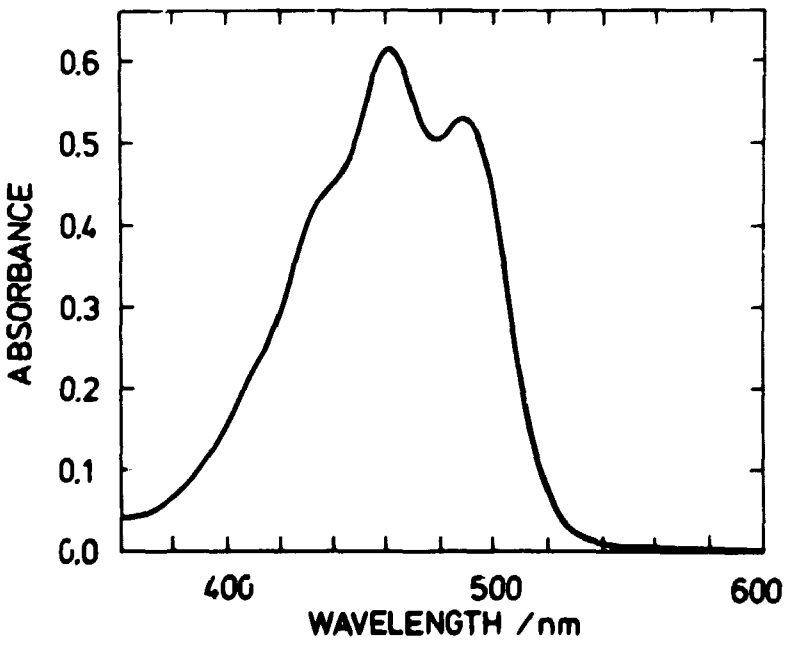


Figure 4. Visible absorption spectrum of all-trans-β-carotene in benzene.

THF used for pulse radiolysis was always prepared immediately before the experiment. All solutions were deoxygenated by bubbling with high purity argon. All irradiations were made at 298±2 K. All transient absorption spectra to be reported are difference spectra.

2.2. Instrumentation

All electron irradiations reported here were performed using the Febetron facility at Risø. The experimental set-up for single- and multi-channel detection, Fig. 5, as it works now, has been described in detail elsewhere (Hansen et al., 1979) and the older facility with single-channel detection only has also been described (Floryan and Pagsberg, 1976; Jensen, 1977). Briefly, optical absorbance changes were obtained by measuring the changes in transmission of light from a monitoring pulsed xenon arc through the sample.

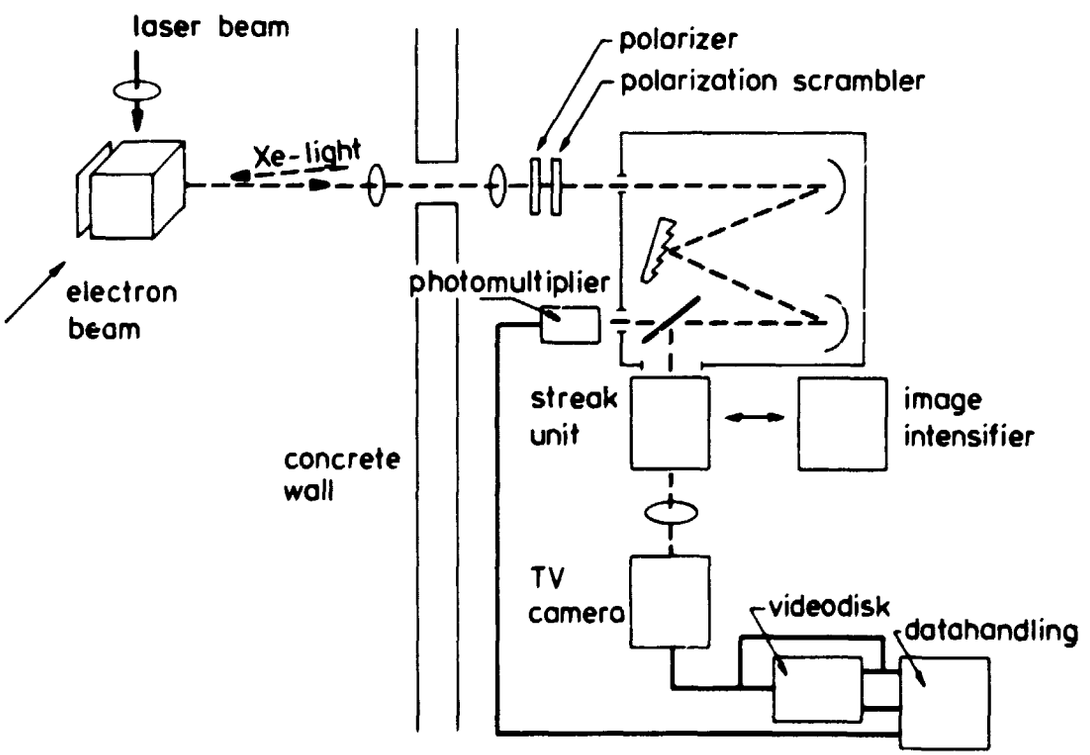


Figure 5. Experimental set-up.

Numerical data on the electron accelerator and dye laser along with a short description of the technique of time-resolved resonance Raman spectroscopy are given in the experimental part of the appendix to the present report.

Computer simulations were made using the program CHEMSIMUL developed by O. Lang Rasmussen (Pagsberg et al., 1977; Hansen et al., 1979). For treatment of the raw kinetic data a computer program was written with which it is possible to plot 1) raw transmission vs. time, 2) absorbance vs. time, 3) log A/A_{max} vs. time, 4) A_{max}/A vs. time and 5) A_{max}/A vs. $\exp(k_1 \cdot t)$ where k_1 must be given. If the products are absorbing at the wavelength of the measurement, the infinity value A_∞ may be subtracted from the measured values of A. Thus, systems with first- and second-order or mixed kinetics may be evaluated with this program.

3. TRIPLET EXCITED STATES

3.1. Introduction

The role of excited triplet states in photosynthesis has been of interest for several years (Livingston, 1960; Robinson, 1963), but only recent experimental results have shown that excited triplet states of polyenes (Witt, 1971) and chlorophylls (Levanon and Norris, 1978; Frank et al., 1979) may be of importance in intact biological systems. The triplet states involving chlorophylls in biological preparations have been directly observed only under conditions where the normal electron transport chain is blocked at a stage immediately after the primary photochemical charge separation. The triplet state of β-carotene, on the other hand, was observed in intact chloroplasts under physiological conditions (Witt, 1971). As the intersystem crossing efficiency of β-carotene is less than 10^{-3} (Bensasson et al., 1977), these observations can be explained only by a sensi-

tized formation of triplet β-carotene, and the sensitizer is most likely chlorophyll a. Furthermore, the absorptions due to carotene triplet states could be observed by excitation at $\lambda > 600$ nm where only the chlorophylls are absorbing. Thus, there is strong indirect experimental evidence for the occurrence of chlorophyll triplet states in biological systems under physiological conditions.

The triplet states observed in these studies are generally not considered as part of the normal photosynthetic reaction chain; rather they are believed to constitute a valve reaction sequence whereby the photosynthetic system is able to channel excess excitation energy away. If the triplet states of the chlorophylls were not quenched rapidly the chlorophyll molecules would be destroyed by oxidation caused by the presence of oxygen.

Whether or not the triplet energy transfer reaction from chlorophyll to β-carotene has other functions than the protective one mentioned remains to be resolved. Triplet β-carotene might have some specialised function in the electron transport, as has been shown for ground-state β-carotene (Tien, 1977) and squalene (Janzen and Bolton, 1979) in studies of model systems. These studies suggested that the alternating polyene structure might facilitate the transmission of electrons through membranes, i.e. function as a "molecular wire". This function depends on extensive overlap within the π-electron framework of the polyenes. In view of the resonance Raman results, to be presented below on triplet β-carotene, which suggest that β-carotene is twisted in the triplet state, this "molecular wire" hypothesis may be extended to include an on/off function depending on whether the polyene is excited (off) or not (on). Alternatively, the triplet energy trapped on β-carotene might be channelled back to the chlorophylls via a triplet-triplet annihilation reaction of β-carotene producing the excited ^1A_g state (Jensen et al., 1980).

With regard to the ground-state carotenoids Beddard et al. (1977) proposed that β-carotene plays a central role in the primary charge separation of photosynthesis. They showed that β-carotene quenched the fluorescence of chlorophyll a with a nearly dif-

fusion-controlled rate constant of $5 \cdot 10^9$ $M^{-1}s^{-1}$. As the singlet energy of β-carotene is higher than that of chlorophyll a, the quenching process probably proceeds by a charge-transfer mechanism generating the radical cation of β-carotene and the radical anion of chlorophyll a. Electron transfer from chlorophyll a to the radical cation of β-carotene has been demonstrated (Lafferty et al., 1976; 1978). Thus, the reaction sequence proposed is

chlorophyll a .... β-carotene .... chlorophyll a $(S_1)^*$

↓

chlorophyll a .... β-carotene$^{+\cdot}$ .... chlorophyll a$^{-\cdot}$

↓

chlorophyll a$^{+\cdot}$ .... β-carotene .... chlorophyll a$^{-\cdot}$

for the primary photochemical charge separation. The mechanism is only speculative as no measurements on biological preparations yet indicate the participation of β-carotene in the primary reaction (Gearle and Wessels, 1978). Carotenoids seem, however, to be integral parts of the reaction centre complexes isolated from photosynthetic bacteria (Feher and Okamura, 1978; Lutz et al., 1978; Agalidis et al., 1980) and green plants (Thornber et al., 1977; Vernon et al., 1971). This infers that the hypothesis cannot be rejected before the role of these carotenoids has been clarified experimentally by studies of purified reaction centre preparations and model systems. In particular these future studies should be aimed at investigations of the possible charge transfer quenching of polyene triplet states and at structural studies by techniques such as time-resolved resonance Raman spectroscopy.

The triplet excited states of the chlorophylls and the polyenes have been studied by flash photolysis (Livingston, 1960; Porter, 1978; Seely, 1978) and pulse radiolysis (Land et al., 1971; Truscott et al., 1973; Bensasson et al., 1977; Bensasson and Land, 1978) and these investigations have provided important information on the spectral properties and chemical reactivities of these species. Important aspects such as the properties

of the chlorophylls as triplet energy acceptors and the quenching of polyene triplet states by charge transfer processes, however, have not been studied previously. Both types of experiments are difficult, if not impossible, to conduct using flash photolysis due to the high extinction coefficients of the chlorophylls and the low intersystem crossing yields of the photosynthetic polyenes.

In flash photolysis (Porter and West, 1974) the primary transient species are the excited states of the light-absorbing substrates; the amount of energy absorbed by a given substrate is determined by the optical absorbance of that particular substrate at the wavelength of the excitation light source. Thus, it is possible to excite a single component selectively in a mixture provided the different components do not have overlapping optical spectra. On the other hand, it may be impossible to excite even the most abundant component selectively in a mixture if the other less abundant components are strongly absorbing compounds.

Conservation of spin angular momentum prohibits optically induced transitions from the singlet ground state to the excited triplet states while the optical transitions to excited singlet states are spin allowed. In the liquid phase, higher excited singlet states generally undergo fast non-radiative relaxation leading to the lowest excited singlet state within a few picoseconds. From this it follows that on a time scale of nanoseconds or longer the outcome of a liquid phase photochemical experiment is largely determined by the photophysical properties of the first excited singlet state. The experiment may, however, be aimed at an investigation of the excited triplet states, in which case the quantum yield of intersystem crossing from the excited singlet state to the triplet state manifold must not be zero or very low if direct excitation of the substrate is to be used.

These two inherent drawbacks of flash photolysis are each important in studies of photosynthetic pigments as mentioned above. In some cases they may be overcome by the use of tunable excitation sources and sensitizers, but generally they will be pro-

hibitive for the two types of experiments mentioned. The last disadvantage of flash photolysis, which is of interest when studying the photophysical properties of excited states, relates to the range of substrate concentrations that may be studied. In order to obtain homogeneous excitation of the sample, the optical absorbance along the exciting light path should be low at the wavelength of the excitation source. If not a concentration gradient of the transient will be produced and the kinetic measurements will not reflect the decay of the transient alone, but the diffusional relaxation of the concentration profile as well. This problem is easily solved by using a tunable excitation source, but in conventional flash photolysis using broad band excitation it generally severely limits the concentration range; for this reason studies of self-quenching, i.e. reactions between excited state and ground state substrate, are impeded. Generally, these reactions can be considered unimportant, but for photosynthetic pigments they may be crucial as the effective pigment concentrations in biological systems are as high as 0.1 M (Sauer, 1978).

By pulse radiolysis (Dorfman, 1974; Spinks and Woods, 1976; Baxendale and Rodgers, 1978) radicals, radical ions and excited states of solute molecules can be produced in differing amounts. The product distribution is determined mainly by the choice of solvent and eventual additives such as N_2O, which in aqueous solution converts the reducing hydrated electron into the potentially oxidizing OH radical.

In protic and polar solvents (water, alcohols, and most ethers except dioxane) ionic species formed by the primary solvent ionization followed by ion solvation and subsequent ion molecule reactions are the main products (Baxendale and Rodgers, 1978). On the other hand, excited states formed by solvent ionization and geminate ion recombination leading to excited states of solvent and solute are the main products in nonpolar hydrocarbon solvents such as benzene and cyclohexane. The physical and chemical mechanisms responsible for ion formation in polar solvents are relatively well described (Symons, 1976; Dainton, 1975; Schindewolf, 1978; Grossweiner, 1978), although the structural

features of solvated electrons still remain obscure. The production of excited states, however, cannot be explained by a unified mechanism as several processes seem to be important (Singh, 1972; Salmon, 1976; Thomas, 1976). The relative importance of the different processes is strongly dependent on the solvent used. In benzene geminate recombination of solvent cations and electrons producing the excited states of benzene followed by energy transfer to the solute appears to be the most important process, while recombination processes involving solute ions operate in cyclohexane (Salmon, 1976). The reported yields, G(S+T), are as high as 5.4 $(100\ eV)^{-1}$ for benzene (Thomas, 1976) and 3.5 $(100\ eV)^{-1}$ for cyclohexane (Salmon, 1976). These conclusions are consistent with the lower yield of free ions in benzene, $G_{fi} = 0.05\ (100\ eV)^{-1}$, compared with cyclohexane where $G_{fi} = 0.15\ (100\ eV)^{-1}$ (Schmidt and Allen, 1968, 1970; Robinson and Rodgers, 1975). The difference between benzene and cyclohexane can be explained qualitatively by the existence in benzene of several excited states of low energy which thermalizes the secondary electrons before they escape too long from the parent positive ions (Cooper and Thomas, 1968). Cyclohexane has no low energy excited states and the electrons have thus a higher probability of escaping their positive counter ions. It was noted above that 1.4-dioxane shows abnormal behaviour as the yield of excited states, $G(S+T) = 2.45\ (100\ eV)^{-1}$, in this ether is considerably higher than in tetrahydrofuran, $G(S+T) = 0.14\ (100\ eV)^{-1}$ (Baxendale et al., 1969, 1970). Again this observation can be correlated with the low yield of free ions, $G_{fi} = 0.046\ (100\ eV)^{-1}$ in 1.4-dioxane compared with $G_{fi} = 0.4\text{-}0.7\ (100\ eV)^{-1}$ for tetrahydrofuran (Schmidt and Allen, 1970; Baxendale et al., 1970; Jou and Dorfman, 1973; Dodelet and Freeman, 1975). In these ethers excited state production due to recombination between solvent cations and solute anions is unlikely as the solvent cations undergo rapid reactions with unionized solvent molecules, whereby the positive charges are lost as protons. In conclusion, the relative yields and/or the relative importance of the different possible processes of excited states seem to be correlated with the yields of free ions in several different systems. Quantitative relations are, however, difficult to establish as neutral radicals produced in the radiolysis

interfere in all the systems mentioned here (see e.g. Salmon et al., 1974).

When comparing pulse radiolysis and flash photolysis the most important difference is that the excitation of solute molecules is an indirect process in pulse radiolysis as described above. Furthermore, the selection rules for optical transitions are generally unimportant in pulse radiolysis because the excited states are produced by ion-recombination processes. Experimentally, this means firstly that the relative amount of excitation in the components of a mixture is determined by the relative molar fractions of the components. Thus, energy transfer to strongly absorbing substrates such as the photosynthetic pigments is easily studied by pulse radiolysis. Secondly, concentrated solutions, which may be optically black in some spectral ranges, can be excited homogeneously producing high yields of triplet states with solutes possessing zero intersystem crossing efficiencies. Hence, pulse radiolysis is a valuable technique in the study of excited states; in this way information, complementary to that obtained by flash photolysis, can be collected. Pulse radiolysis results should, however, be compared with those of flash photolysis wherever possible, as the mode of excitation is less "clean" in pulse radiolysis relative to flash photolysis.

In the following paragraphs of this chapter the results obtained by naphthalene- sensitized production of triplet chlorophyll a and β-carotene will be presented along with data obtained on triplet duroquinone. Related results obtained on the three systems will be discussed separately. Finally, the use of the new technique time-resolved resonance Raman spectroscopy on excited states of photosynthetic pigments will be introduced and the results will be presented.

3.2. Time-resolved absorption spectroscopy and kinetics

3.2.1. The sensitizer: naphthalene

Naphthalene has been used in these studies as a triplet sensitizer in the formation of triplet chlorophyll a and β-carotene.

Naphthalene was chosen as sensitizer because the formation and decay of triplet naphthalene by pulse radiolysis of naphthalene in benzene have been the subject of a number of previous investigations (Cooper and Thomas, 1968; Baxendale and Fiti, 1972; Dainton et al., 1972; Singh, 1972; Salmon, 1976). These investigations have provided spectral and kinetic data of the triplet state of naphthalene, but it was necessary to reinvestigate this system under the present experimental conditions as the first-order rate constant for decay of triplet naphthalene was found previously to be dose dependent. Furthermore, measurements on the naphthalene system could give a calibration of the doses delivered by the Febetron accelerator.

Pulse radiolysis of a 10^{-2} M Ar-saturated solution of naphthalene in benzene gave rise to a transient absorption with λ_{max} = 420 nm and 400 nm in agreement with previously reported results (Dainton et al., 1972; Porter and Wilkinson, 1961). The transient absorbance at 420 nm increased linearly with dose within the range of doses studied indicating that the trapping of triplet energy by naphthalene competes efficiently with the other decay channels of the primary excited states produced in the system. Using the value $G \cdot \Delta\varepsilon = 3.58 \cdot 10^4$ $(100\ eV)^{-1}$ $M^{-1}cm^{-1}$ for the triplet yield inferred from Dainton et al. (1972), the absorbance vs. relative dose curve is used to determine the effective absolute dose corresponding to a given relative dose. In this way the relation: 1 relative dose unit = 3.33 krad was obtained.

The decay of triplet naphthalene followed first-order kinetics at low relative doses but at higher doses a clear second-order component was visible in the plots of log (A/A_{mx}) vs. time. Furthermore, the rate constants for the first-order component increased with increasing dose. Thus, in order to characterize the system it was necessary to evaluate the rate constants at different relative irradiation doses.

For a transient species decaying by mixed first- and second-order reactions the following expression for the time dependence of the transient absorbance is obeyed (Benson, 1960), provided that

no absorbing products are produced

$$\frac{A_{max}}{A} = \left(1 + \frac{2k_2 A_{max}}{k_1 \cdot \Delta\varepsilon \cdot \ell}\right) \exp(k_1 t) - \frac{2k_2 A_{max}}{k_1 \cdot \Delta\varepsilon \cdot \ell}$$

where A, $\Delta\varepsilon$ and ℓ refer to the absorbance, the difference extinction coefficient and the optical path length, respectively. Rate constants k_1 and k_2 are defined by the reaction scheme below.

SCHEME

$$\begin{aligned}
&Bz && \rightarrow && Bz^+, e^-, Bz^S, Bz^T && \\
&Bz^+ + e^- && \rightarrow && Bz^S, Bz^T && \\
&Bz^S && \rightarrow && Bz, Bz^T \ (+ h\nu_f) && \\
&Bz^S + N && \rightarrow && Bz + N^S \rightarrow N + N^T \ (+ h\nu_f) && \\
&Bz^T + N && \rightarrow && Bz + N^T && \\
&N^T && \rightarrow && N && k_1 \\
&2N^T && \rightarrow && 2N && 2k_2 \\
&N^T + R\cdot && \rightarrow && N + R\cdot && k_3
\end{aligned}$$

where Bz is benzene, N is naphthalene, and R· is a radiolytically produced radical derived from benzene. Superscripts S and T denote the excited singlet and triplet states, respectively.

Thus $2k_2/\Delta\varepsilon$ may be determined from plots of A_{max}/A vs. $\exp(k_1 t)$ if k_1 has been measured independently. The values of k_1 were determined from the limiting slopes of the plots of log (A/A_{max}) vs. time. Figure 6 shows an example of this analysis. As indicated in Fig. 7 the first-order rate constant increases with dose while the second-order rate constant is independent of dose.

The dose dependence of the first-order rate constant has been explained by enhanced spin inversion induced by paramagnetic species produced during the radiation pulse (Dainton et al.,

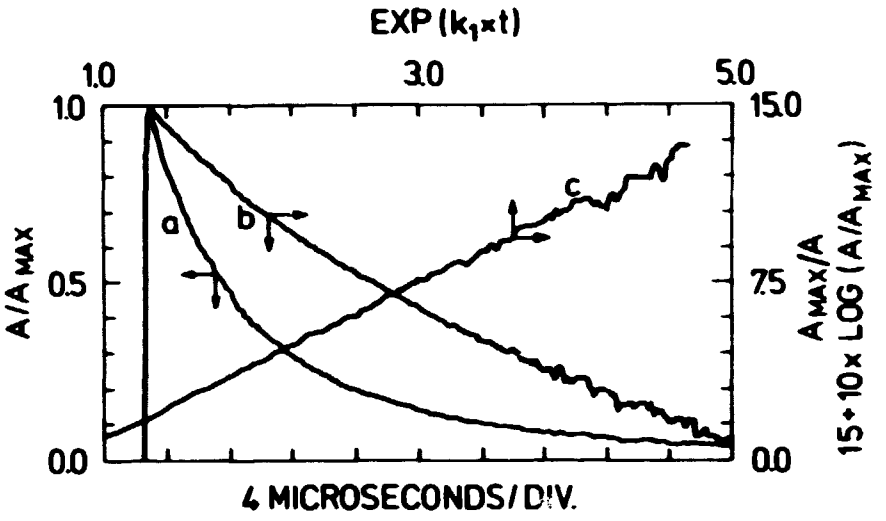


Figure 6. Analysis of the decay of triplet naphthalene (420 nm) obtained by pulse irradiation of 10^{-2} M naphthalene in benzene with a dose of 7.6 krad. a) Plot of A/A_{max} versus time. A_{max} = 0.652 at optical path length of 2 cm. b) Plot of log (A/A_{max}) versus time giving k_1 as the limiting slope. c) Plot of A_{max}/A versus $\exp(k_1 \cdot t)$. Time t is measured relative to t_{max} defined by $A = A_{max}$ for $t = t_{max}$. $2k_2/\Delta\varepsilon$ is calculated from the slope of the line.

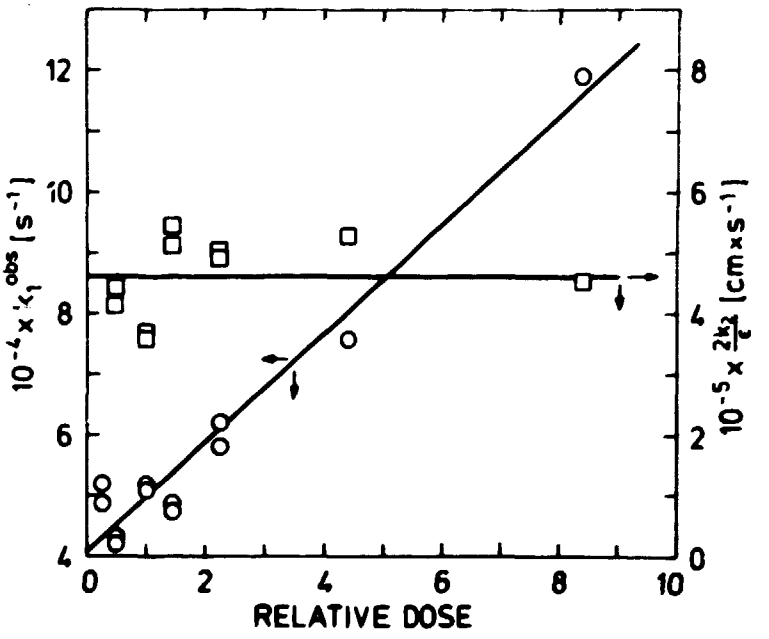


Figure 7. Plots of k_1 and $2k_2/\Delta\varepsilon$ for the decay of triplet naphthalene in benzene versus the relative irradiation dose. The mean value of $2k_2/\Delta\varepsilon$, $(4.61 \pm 0.65) \cdot 10^5$ cm·s^{-1}, is indicated by the line.

1972). From Fig. 7 a slope of $2.66 \cdot 10^{3}$ s^{-1} $krad^{-1}$ is obtained for the dose dependence of the first-order rate constant. In calculating this value the relation between relative and absolute dose given above has been used. The yield of radicals produced by radiolysis of benzene has been reported as G = 0.78 $(100\ eV)^{-1}$ (Hardwick, 1966). Using this value an apparent bimolecular rate constant $k_3 = 3.7 \cdot 10^{9}$ $M^{-1}s^{-1}$ can be calculated for the quenching of the naphthalene triplet state by the radiation-induced free radicals. A possible quenching mechanism is a catalytic quenching process (Porter and Wright, 1959) whereby the quencher gets its unpaired spin reversed only by interaction with the triplet naphthalene. The triplet naphthalene, in turn, undergoes intersystem crossing from the triplet to the singlet manifold:

$$N(\uparrow\uparrow)^{*} + R(\uparrow) \rightarrow N(\uparrow\downarrow)^{*} + R(\downarrow) \rightarrow N(\uparrow\downarrow) + R(\downarrow)$$

where $N(\uparrow\uparrow)^{*}$ represents triplet excited naphthalene, $N(\uparrow\downarrow)^{*}$ excited naphthalene in the singlet manifold, $N(\uparrow\downarrow)$ ground state naphthalene, and $R(\uparrow)$ the radiation-induced free radical which acts as quencher. A spin-exchange reaction of this type generally requires the formation of an intermediate encounter complex (Lamola, 1969). Hence, omitting spin statistical factors, the upper limit for the observed bimolecular rate constant is given by the diffusion-limited rate constant (Weston and Schwarz, 1972) for reactions between two different species:

$$k_D = 4\pi(D_A+D_B)\ R_{AB}\ \frac{N_o}{1000}$$

where D_A and D_B are the diffusion coefficients of the reactants, R_{AB} is the contact radius of the reactants, and N_o is Avogadro's number.

Using the Stokes-Einstein relation

$$D_i = \frac{kT}{6\pi\eta r_i}$$

where η is the macroscopic viscosity of the medium and r_i is the radius of the species, we get

$$k_D = \frac{2RT}{3000\ \eta}\left(\frac{(r_A+r_B)^2}{r_A r_B}\right)$$

The effective radii r_A and r_B are not known but setting $r_A = r_B$ gives the well-known relation

$$k_D = \frac{8RT}{3000\ \eta}$$

The value $k_D = 1.1 \cdot 10^{10}\ M^{-1}s^{-1}$ is calculated for benzene at T = 298 K.

In order to obtain the true upper limit this value should be corrected for the spin statistics involved:

$$N(S=1) + R(S = \tfrac{1}{2}) \rightarrow \begin{cases} [N \ldots R](S = 1\tfrac{1}{2}) \rightarrow N(S = 1) + R(S = \tfrac{1}{2}) \\ [N \ldots R](S = \tfrac{1}{2}) \rightarrow N(S = 0) + R(S = \tfrac{1}{2}) \end{cases}$$

where [N ... R] represents the encounter complex.

Formation of the reactive $S = \frac{1}{2}$ encounter complex has a probability of 1/3 due to spin statistics alone. The encounter complex with $S = 1\frac{1}{2}$ cannot be reactive if the possibility of energy and charge transfer is excluded. Thus, the spin-corrected upper limit for the observed rate constant is given by $\frac{1}{3} k_D = 3.7 \cdot 10^9\ M^{-1}s^{-1}$. The agreement between the values may be fortuitous but it should be noticed that the observed dose dependence of the first-order rate constant of triplet duroquinone gives a similar value of the apparent bimolecular rate constant.

The possibility of self-quenching of the naphthalene triplet has not been investigated, but the decay has previously been found to be independent of the concentration of ground-state naphthalene (Singh, 1972).

The results obtained here agree well with the observations of Dainton et al. (1972). These workers probably used an accelerator operating at a constant dose rate, and the dose was varied by altering the pulse length. Our accelerator, on the other hand, operates at a constant pulse length and the dose is varied by means of attenuators inserted into the beam. Thus, the effective dose rate on the sample is varied. This difference in the experimental methods may explain the difference in the observed dose dependence. Dainton et al. (1972) found a stronger dose dependence of the first-order rate constant, as they reported $k_1 = 3.2 \cdot 10^4\ s^{-1}$ at the low dose limit compared with our value $k_1 = 4.1 \cdot 10^4\ s^{-1}$. At high dose, Dainton et al. (1972) reported $k_1 = 20.7 \cdot 10^4\ s^{-1}$ at 14.8 krad compared with $k_1 = 12 \cdot 10^4\ s^{-1}$ at 28 krad found in this work. The value of $2k_2/\Delta\varepsilon = (4.61 \pm 0.65) \cdot 10^5$ cm s^{-1} determined in this work is identical to the benzene value reported by Dainton et al. (1972); this result shows that k_2 is independent of dose as well as dose rate. This is to be expected because the triplet-triplet annihilation process should be independent of the mode of production as long as the triplet-excited species are homogeneously distributed and no other apparent second-order reactions contribute to the observed decay.

The second-order rate constant for the decay of triplet naphthalene in benzene has apparently not been measured by flash photolysis, but Dainton et al. (1972) noted that their value in cyclohexane compares well with those of previous flash photolysis experiments in the same solvent.

The mechanism of the triplet-triplet annihilation of naphthalene has been discussed elsewhere (Tfibel and Lindqvist, 1975) and it should be noticed that excited singlet-state production appears to be very effective. The experimental singlet yield, p_e, defined by

$$\gamma_s = p_e k \ ; \quad k = 2k_2 \ ,$$

where γ_s is the rate constant of excited singlet formation, has been determined to $p_e = 0.52$ in cyclohexane by time-resolved measurements of delayed fluorescence and triplet absorption

(Tfibel and Lindqvist, 1975). When p_e is defined in this way, the maximum theoretical value is $p_e = 0.5$. Hence, the results of Tfibel and Lindqvist (1975) indicate in fact that excited singlet states are formed with 100% efficiency in the triplet-triplet annihilation reaction of triplet-excited naphthalene. It seems reasonable to assume that this result holds under our conditions, too.

In conclusion the kinetic properties of the triplet state of naphthalene have been characterized. Thus, these data can be used in the evaluation of the sensitized formation of triplet-excited chlorophyll a and β-carotene to be described below.

3.2.2. Chlorophyll a

This study was initiated in order to investigate triplet energy transfer from a sensitizer (naphthalene) to chlorophyll a and to record the time-resolved resonance Raman spectrum of triplet-excited chlorophyll a.

Pulse irradiation of a Ar-saturated benzene solution containing 10^{-2} M naphthalene and $5.6 \cdot 10^{-5}$ M chlorophyll a gave a strong transient absorption with $\lambda_{max} = 470$ nm and a long tail beyond 560 nm, as shown in Fig. 8. This transient absorption is assigned to the lowest triplet state of chlorophyll a on the basis of its mode of production and its spectral resemblance to the photolytically prepared triplet state (Linschitz and Sarkanen, 1958). In order to establish that the observed signals were not distorted by light emission during and after the irradiation pulse (other than the normal Cerenkov emission), the light reaching the photomultiplier with the xenon lamp turned off was monitored. Under the experimental conditions used for absorbance measurements no emission from the sample could be detected. Thus, the observed optical absorbances needed no correction due to light emission.

In order to describe the system kinetically the dose dependence of the transient absorption was investigated at various concen-

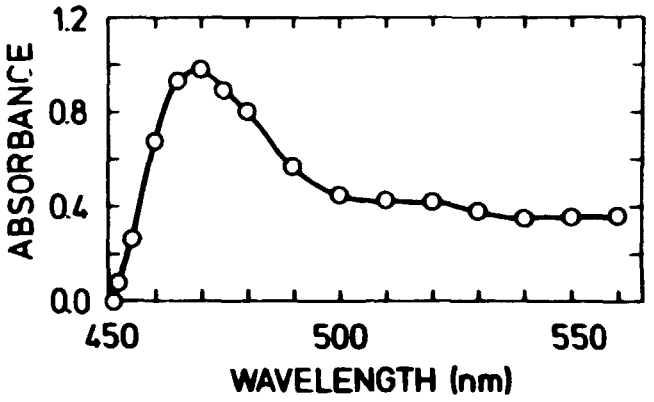


Figure 8. Transient difference absorption spectrum of the lowest excited triplet state of chlorophyll a obtained by pulse irradiation of $5.6 \cdot 10^{-5}$ M chlorophyll a in benzene containing 10^{-2} M naphthalene. Dose = 41 krad. Path length = 2 cm.

trations of chlorophyll a but at a constant (10^{-2} M) concentration of naphthalene.

The plots of transient maximum optical density versus relative doses were curved, except at the highest chlorophyll concentrations. This indicated a relatively low rate constant for energy transfer from naphthalene to chlorophyll a compared with the rate constants for decay of triplet naphthalene. The results were analysed on the basis of the general scheme for triplet-triplet energy transfer:

N	N^T	Excitation (see scheme in 3.2.1)
N^T	$\rightarrow N$	k_1
$N^T + N^T$	$\rightarrow 2N$	$2k_2$
$N^T + Chl$	$\rightarrow N + Chl^T$	k_4
Chl^T	$\rightarrow Chl$	k_5
$Chl^T + Chl^T$	$\rightarrow 2Chl$	$2k_6$

where N = ground-state naphthalene, N^T = triplet naphthalene,

Chl = ground-state chlorophyll *a* and Chl^T = triplet chlorophyll *a*.

As the rate of formation of triplet chlorophyll appeared to be rapid compared with its rate of decay except at the lowest concentrations of chlorophyll, we may assume as a first approximation that triplet chlorophyll is stable within its time of formation. In this case the yield of triplet chlorophyll expressed in terms of the primary available triplet naphthalene is given by the following equations:

$$-\frac{d(N^T)}{dt} = k_1(N^T) + 2k_2(N^T)^2 + k_4(N^T)(Chl)$$

$$\frac{d(Chl^T)}{dt} = k_4(N^T)(Chl)$$

which leads to

$$-d(Chl^T) = \frac{k_4(Chl)}{k_1 + k_4(Chl) + 2k_2(N^T)}\, d(N^T)$$

By integration from $(N^T) = (N^T)_{max}$ to $(N^T) = 0$ one obtains

$$(Chl^T)_{max} = \frac{k_4(Chl)}{2k_2} \cdot \ln\left(1 + \frac{2k_2(N^T)_{max}}{k_1 + k_4(Chl)}\right)$$

The term $\frac{2k_2(N^T)_{max}}{k_1 + k_4(Chl)}$ can be estimated to take values between 0 and 1 for the systems studied here. Thus, the following expansion of the logarithmic function, retaining only the first term, is justified. Using

$$\ln(1+x) = x - \frac{1}{2}x^2 + \ldots \qquad \text{for } x < 1$$

we obtain

$$(Chl^T)_{max} = \frac{k_4(Chl)}{k_1 + k_4(Chl)} \cdot (N^T)_{max}$$

or

$$\frac{1}{(Chl^T)_{max}} = \frac{1}{(N^T)_{max}} \left(1 + \frac{k_1}{k_4(Chl)}\right)$$

By using A_{max}^T for the maximum absorbance of triplet chlorophyll, $\Delta\varepsilon_{470}^{Chl^T}$ for the difference extinction coefficient of triplet chlorophyll at 470 nm, and ℓ for the optical path length (2 cm) we finally obtain

$$\frac{1}{A_{max}} = \frac{1}{(N^T)_{max} \cdot \Delta\varepsilon_{470}^{Chl^T} \cdot \ell} \left(1 + \frac{k_1}{k_4(Chl)}\right)$$

Within this approximation a plot of $1/A_{max}$ versus $1/(Chl)$ should give straight lines for each irradiation dose, with

$$\text{intercept} = \frac{1}{(N^T)_{max} \cdot \Delta\varepsilon_{470}^{Chl^T} \cdot \ell}$$

$$\text{slope} = \frac{1}{(N^T)_{max} \cdot \Delta\varepsilon_{470}^{Chl^T} \cdot \ell} \cdot \frac{k_1}{k_4}$$

As mentioned above these equations are valid only if the triplet state of chlorophyll a is stable within its time of formation. This is not the case under our conditions. Thus, the measured values of A_{max} are too low. The experimental values of A_{max} are, however, easily corrected for decay during the formation if only contributions from first-order processes are taken into account (Amouyal et al., 1974). The relation used is

$$A_{max}^{corr} = A_{max} \cdot \exp\left(\frac{\ln k_D/k_A}{k_D/k_A - 1}\right)$$

where $k_D = k_1 + k_4(Chl)$ and $k_A = k_5 \cdot k_1$ and k_5 are known at all concentrations and doses, but k_4 is unknown. From plots of the uncorrected values of $1/A_{max}$ vs. $1/(Chl)$ we were able to estimate that $1.5 \cdot 10^9\ M^{-1}s^{-1} < k_4 < 4.5 \cdot 10^9\ M^{-1}s^{-1}$. Thus $k_4 = 3 \cdot 10^9\ M^{-1}s^{-1}$ is a reasonable value to use in the correction of the measured values of A_{max}. The correction amounted to ca. 85% at the lowest

concentrations of chlorophyll and ca. 15% at the highest concentrations. Figure 9 shows the plot of $1/A_{max}^{corr}$ vs. 1/(Cl) along with the least-square-fitted lines.

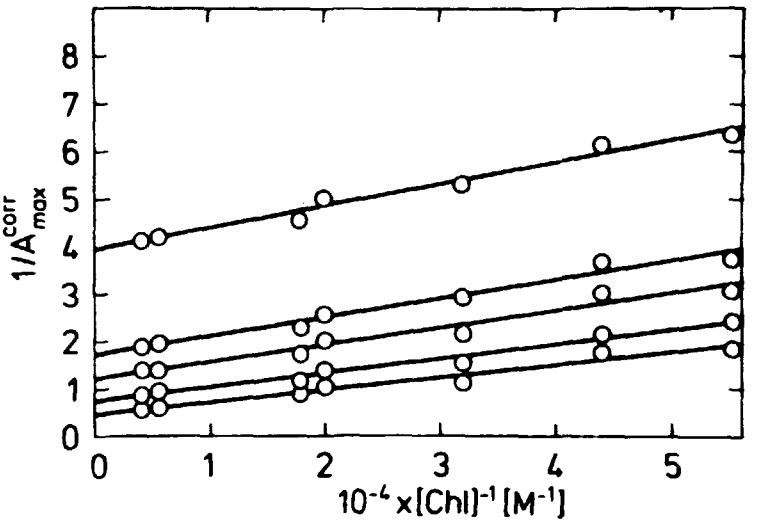


Figure 9. Plot of the corrected reciprocal maximum absorbance, $1/A_{max}^{corr}$, of triplet chlorophyll a versus the reciprocal concentration of chlorophyll a, 1/[Chl], at various irradiation doses. Solvent: benzene containing 10^{-2} M naphthalene. Path length = 2 cm. Relative doses going from the upper to the lower line are 0.49, 1.0, 1.44, 2.25 and 4.41 respectively. Extinction coefficients and rate constants are calculated from the intercept and slope of the least-square lines drawn.

As $(N^T)_{max}$ and k_1 have been measured independently, we are able to determine $\Delta\varepsilon_{470}^{Chl^T}$ and k_4 from the slopes and intercepts of Fig. 9. Table I summarizes the information obtained from Fig. 9. The values of $\Delta\varepsilon_{470}^{Chl^T}$ are well determined, as the intercepts of Fig. 9 are insensitive to the corrections of A_{max}. On the other hand, Table I shows that the apparent values of k_4 decrease with increasing dose. This trend is probably due to the neglect of all second-order processes in the correction of the measured values of A_{max}. Thus, the value of k_4 determined at relative dose 0.49 should be close to the true value, as second-order processes are unimportant at this dose. This possibility has been tested by computer simulation of the complete kinetic system including first- and second-order decay of triplet chlorophyll a. Examples of experimental kinetic curves and model

Table I

Dose[a]	Slope[b] 10^{-5} M	Intercept[b]	$\varepsilon_{470}^{Chl^T}$ 10^4 $M^{-1}cm^{-1}$	k_4 from Fig. 9 10^9 $M^{-1}s^{-1}$	k_4[c] simulation 10^9 $M^{-1}s^{-1}$
0.49 (1.7)	4.57	3.93	3.2	3.9	3.5±0.4
1.0 (3.4)	3.91	1.73	3.8	2.2	3.6±0.6
1.44 (4.9)	3.54	1.21	3.5	1.8	3.6±0.6
2.25 (7.6)	3.03	0.730	3.3	1.5	3.8±0.6
4.41 (14.7)	2.51	0.459	3.2	1.4	-

a) Values in parentheses are doses in krad.

b) From Fig. 9.

c) These values are mean values of rate constants obtained by simulation of experimental decay curves for [Chl] = $3.13 \cdot 10^{-5}$ M, $5.03 \cdot 10^{-5}$ M and $10.1 \cdot 10^{-5}$ M.

curves are shown in Fig. 10, and the values of k_4 determined by the simulations are included in the last column of Table I. These computer simulations included the first-order rate constants for decay of triplet chlorophyll determined experimentally in this work and an estimate of the second-order rate constant for decay of triplet chlorophyll. The latter constant $2k_6 = (1.4 \pm 0.3) \cdot 10^9$ $M^{-1}s^{-1}$ is somewhat lower than the value $2.1 \cdot 10^9$ reported by Linschitz and Sarkanen (1958).

The first-order rate constants for decay of triplet chlorophyll determined in this work showed only a slight dependence upon irradiation dose. At all concentrations the low dose limit, however, was the same, $k_5 = (2.2 \pm 0.3) \cdot 10^4$ s^{-1}. This value of the first-order rate constant is ca. 50 times greater than the one reported by Linschitz and Sarkanen (1958). Part of this discrepancy may be due to the difference in technique, as triplet states produced by pulse radiolysis commonly have shorter life-times than when produced by flash photolysis (Singh, 1972).

No systematic variation of k_5 with the concentration of chlorophyll ($1.81 \cdot 10^{-5}$ M – $2.48 \cdot 10^{-4}$ M) was detected, indicating that $k_5' < 5 \cdot 10^7$ $M^{-1}s^{-1}$ where k_5' is the rate constant for quenching triplet chlorophyll by ground-state chlorophyll:

$$Chl^T + Chl \rightarrow 2Chl \qquad k_5'$$

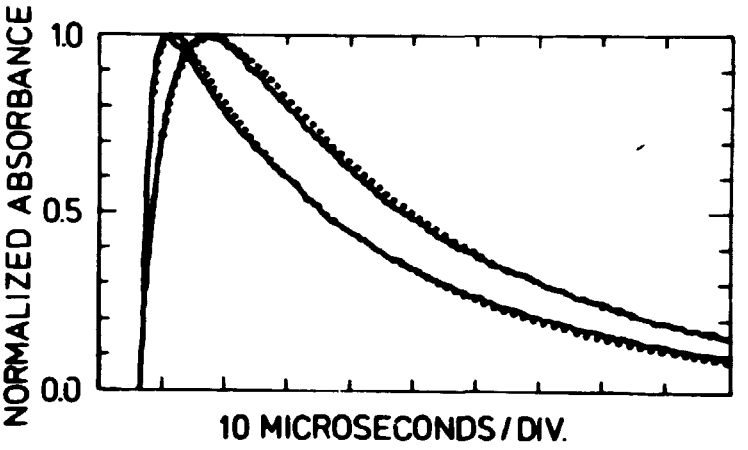


Figure 10. Experimental (full lines) and calculated (dotted lines) formation and decay curves (470 nm) for triplet chlorophyll a in benzene containing 10^{-2} M naphthalene. The irradiation dose was constantly 3.4 krad and the bulk chlorophyll a concentrations were $3.13 \cdot 10^{-5}$ M (slow formation) and $18.1 \cdot 10^{-5}$ M (fast formation). Rate constants k_1 and $2k_2$ were taken from Fig. 7. Values of k_5 were the experimental values $2.3 \cdot 10^4$ s^{-1} (slow) and $2.6 \cdot 10^4$ s^{-1} (fast). The remaining rate constants and extinction coefficient were $k_4 = 4.3 \cdot 10^9$ $M^{-1}s^{-1}$, $2k_6 = 1.8 \cdot 10^9$ $M^{-1}s^{-1}$, $\Delta\varepsilon_{470} = 3.3 \cdot 10^4$ $M^{-1}cm^{-1}$ at [Chl] = $3.13 \cdot 10^{-3}$ M and $k_4 = 3.1 \cdot 10^9$ $M^{-1}s^{-1}$, $2k_6 = 1.0 \cdot 10^9$ $M^{-1}s^{-1}$, $\Delta\varepsilon_{470} = 3.5 \cdot 10^4$ $M^{-1}cm^{-1}$ at [Chl]= $18.1 \cdot 10^{-5}$ M.
Curves were normalized at $A_{max} = 0.242$ ($3.13 \cdot 10^{-5}$ M) and $A_{max} = 0.444$ ($18.1 \cdot 10^{-5}$ M).

Linschitz and Sarkanen (1958) originally determined $k_5' = 5 \cdot 10^7$ $M^{-1}s^{-1}$ but later this result was questioned (Linschitz et al., 1962) as the apparent self-quenching might be due to impurities introduced at high solute concentrations.

3.2.3. All-trans-β-carotene

Pulse irradiation of a Ar-saturated benzene solution containing 10^{-2} M naphthalene and 10^{-4} M β-carotene gave an extremely strong transient absorption with maximum at 530 nm. The absorption spectrum shown in Fig. 11 agrees well with previously published triplet-triplet absorption spectra of β-carotene (Land et al., 1971; Mathis and Kleo, 1973). Furthermore, the present data confirm that the absorption has a maximum at ca. 530 nm in benzene (Mathis and Kleo, 1973) compared with 515 nm in hexane (Chessin et al., 1966; Land et al., 1971). The same transient

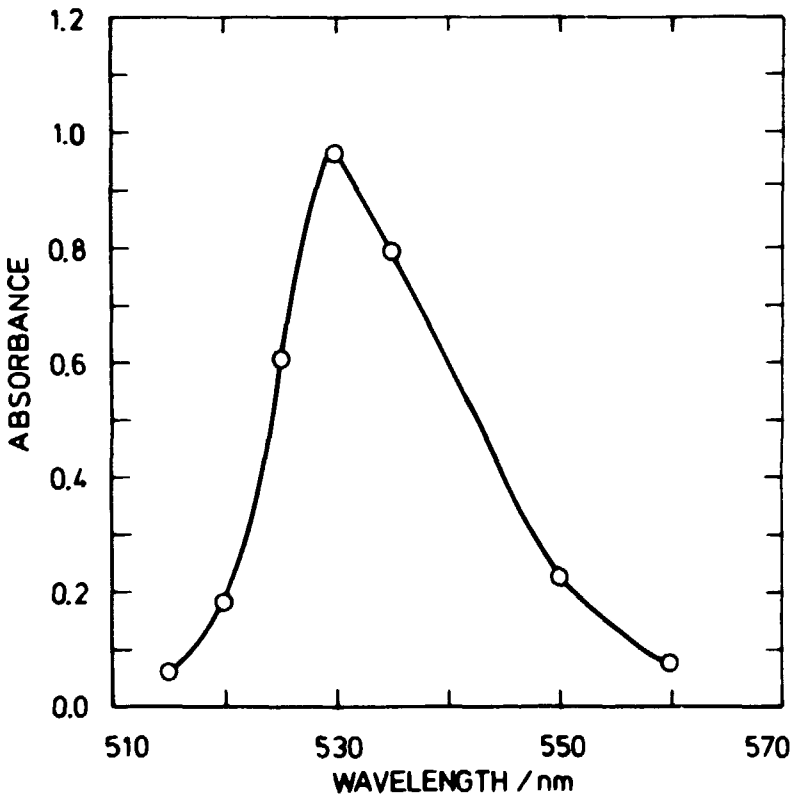


Figure 11. Transient difference absorption spectrum of the lowest excited triplet state of all-trans-β-carotene obtained by pulse irradiation of 10^{-4} M all-trans-β-carotene in benzene containing 10^{-2} M naphthalene. Dose = 1.7 krad. Optical path length = 2 cm.

absorption with λ_{max} = 530 nm was observed using 10^{-2} M biphenyl as sensitizer, thus supporting the assignment of the absorption to the lowest triplet state of β-carotene. However, the yield of triplet β-carotene was approximetely 2 times lower with biphenyl than with naphthalene. This reduction of the yield probably reflects a lower efficiency of energy transfer to β-carotene compared with the intrinsic decay of the sensitizer for biphenyl relative to naphthalene.

The transient optical absorbance measured at 530 nm and 560 nm increased linearly with dose even at the highest doses used, indicating a highly efficient triplet-triplet energy transfer process between naphthalene and β-carotene. Figure 12 shows the transient optical absorbance over 2 cm as a function of

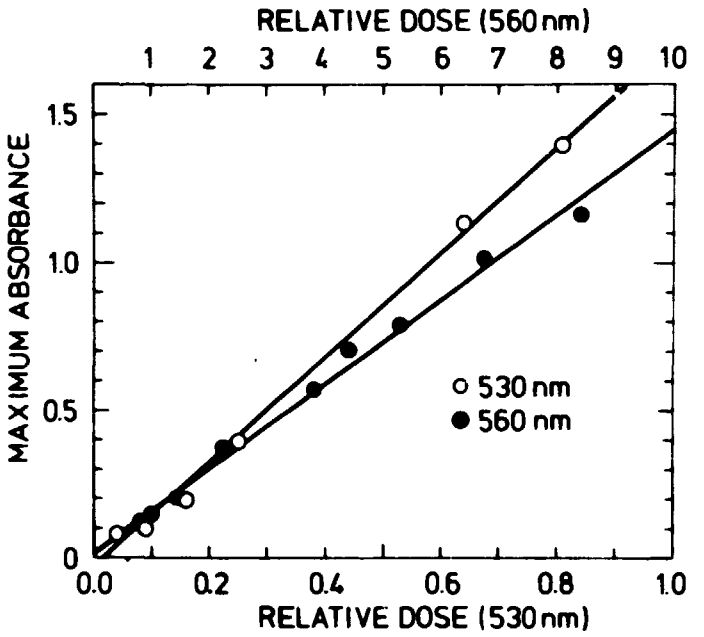


Figure 12. Plot of maximum transient absorbance at 530 nm and 560 nm versus relative irradiation dose. System: 10^{-4} M β-carotene in benzene containing 10^{-2} M naphthalene. Optical path length = 2 cm. The lines drawn were obtained by least-square analysis of the experimental points.

relative dose. The ratio of the slopes of the least-square lines gives the ratio of the extinction coefficient at 530 and 560 nm as $\Delta\varepsilon_{530}/\Delta\varepsilon_{560} = 12.4 \pm 1.2$. This value along with the $\Delta\varepsilon_{max} = 2.38 \cdot 10^{5}$ $M^{-1}cm^{2}$ reported by Bensasson et al. (1977) will be used later in the determination of the rate constants involved.

Although the rate of triplet energy transfer from naphthalene appeared to be extremely high the use of the method of analysis employed in the chlorophyll system is unjustified. The reason for this is that the decay of triplet β-carotene under our experimental conditions was approximately 10 times faster than that of triplet chlorophyll. Thus, computer simulation of the complete kinetic system is the only meaningful method for extracting the rate constants which cannot be obtained by other means.

The system was analyzed on the basis of a scheme identical with that given for chlorophyll, i.e.:

$$N^T + C \rightarrow N + C^T \qquad k_7$$

$$C^T \rightarrow C \qquad k_8$$

$$C^T + C^T \rightarrow 2C \qquad 2k_9$$

where C = ground state β-carotene and C^T = triplet β-carotene. The rate constants for the decay of triplet naphthalene have been determined above. The first-order rate constants for the decay of triplet β-carotene were determined as the limiting slopes of logarithmic plots of absorbance versus time. These rate constants were independent of dose as shown in Fig. 13; the mean value is $k_8 = (2.9 \pm 0.29) \cdot 10^5\ s^{-1}$. At high doses the

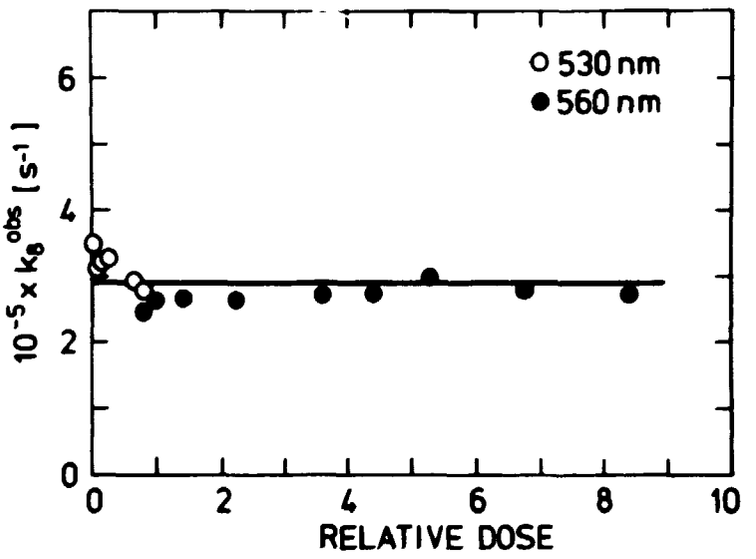


Figure 13. Plot of the experimental first-order rate constant, k_8, for the decay of triplet β-carotene versus the relative irradiation dose. Data at low and high doses were obtained at 530 nm and 560 nm respectively. The mean value, $2.9 \cdot 10^5\ s^{-1}$, is indicated by the full line.

logarithmic plots of absorbance versus time showed some curvature indicating a contribution from the triplet-triplet annihilation reaction included in the reaction scheme. This reaction has not been reported previously, probably because the curvature is detectable only at high doses due to interference from

the overlapping curvature produced by the formation reaction. Thus, only the rate constants for triplet-triplet energy transfer and triplet-triplet annihilation remain to be determined. Using t_{max}, $t_{\frac{1}{2}}$, and A_{max} as the experimental quantities to be fitted the results summarized in Table II were obtained. Figure 14 shows an experimental decay curve plotted together with the computer-simulated curve. The rate constants obtained are $k_7 = (10.7 \pm 1.2) \cdot 10^9$ $M^{-1}s^{-1}$ and $2k_9 = (3.6 \pm 0.4) \cdot 10^9$ $M^{-1}s^{-1}$.

Table II. Rate constants for triplet ß-carotene from computer simulation

Dose krad	$A^{exp.}_{max}$ 560 nm	$A^{calc.}_{max}/A^{exp.}_{max}$ [a] 560 nm	k_7 10^9 M^{-1} s^{-1}	$2k_9$ 10^9 $M^{-1}s^{-1}$
2.8	0.128	1.09	8.5	4.4
7.6	0.373	1.00	10.2	4.0
12.1	0.570	1.00	10.8	3.2
14.7	0.706	0.96	11.0	3.2
17.6	0.790	1.00	11.5	3.6
22.5	1.015	0.96	12.0	3.2

a) Calculated using $\Delta\varepsilon_{530} = 2.38 \cdot 10^5$ $M^{-1}cm^{-1}$ and $\Delta\varepsilon_{530}/\Delta\varepsilon_{560} = 12.4$.

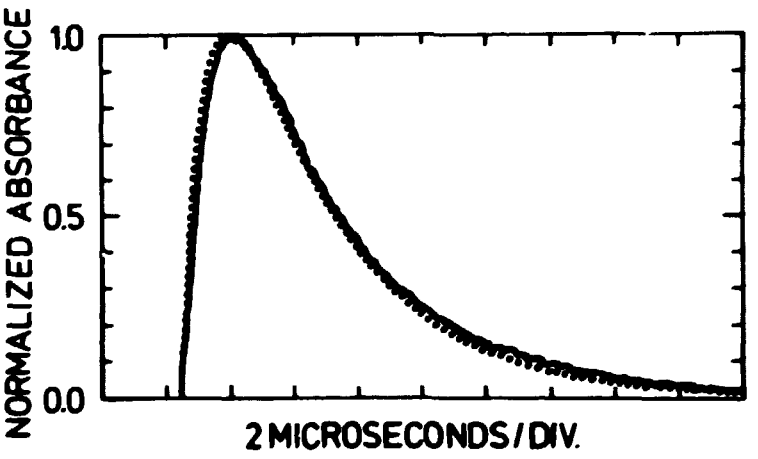
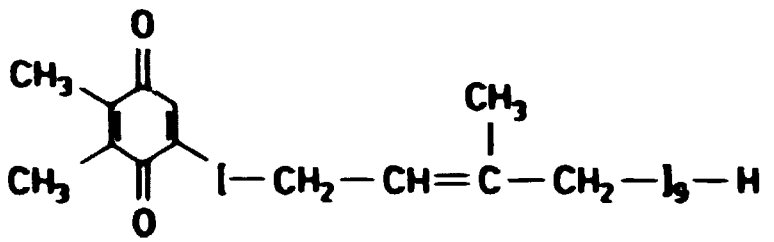


Figure 14. Experimental (full line) and calculated (dotted line) formation and decay curve (560 nm) for triplet ß-carotene in benzene containing 10^{-2} M naphthalene. The irradiation dose was 7.6 krad and the bulk concentration of ß-carotene 10^{-4} M. Rate constants k_1 and k_2 were taken from Fig. 7 and k_8 from Fig. 13. $\Delta\varepsilon_{560}$ was fixed at $1.92 \cdot 10^4$ M^{-1} cm^{-1}. The values fitted for k_7 and $2k_9$ are shown in Table II.

3.2.4. Duroquinone

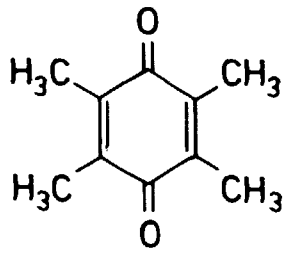
An important part of the coupling chain between photosystem II and I in the photosynthesis of green plants is the pool of plastoquinone, which acts as an electron buffer and connects several reaction centers in the photosynthetic membrane (Amesz, 1977).

PLASTOQUINONE



Furthermore, the plastoquinone pool takes part in the non-cyclic production of ATP, as electron transfer from plastoquinone to cytochrome f is coupled to phosphorylation of ADP. The processes involved are intimately related to the membrane structure of the photosynthetic system and they have been discussed by Jagendorf (1975) and Witt (1975).

Although plastoquinone is probably not directly reduced by photo-excited chlorophyll in photosynthesis, processes involving photo-induced charge transfer from chlorophylls to quinones have been studied extensively (Seely, 1978; Porter, 1978) in attempts to model photosystem II. Photosystem II, which produces molecular oxygen and reduced quinone, might provide a cyclic process for storage of solar energy, as hydroquinone can be reacted with oxygen or be used for production of molecular hydrogen. Because of its structural similarity with plastoquinone, duroquinone has been one of the model compounds mostly used in these studies. None of these studies, however, have involved intermediate excited states of the quinones, as the light energy has been absorbed entirely by the chlorophylls involved.



DUROQUINONE

Another approach to the problem of photochemical solar energy storage has been the use of duroquinone as the light-absorbing dye. These studies have been prompted by the high oxidizing power of duroquinone in its lowest excited triplet state (Scheerer and Grätzel, 1977; Meisel and Czapski, 1975)

$$E^{o}(DQ^{T}/DQ^{-}) \approx E^{o}(DQ/DQ^{-}) + E_{T} = +\ 2.2V \text{ (vs. NHE)}$$

where E^{o} is the redox potential of the redox couple in parenthesis and E_{T} is the triplet energy. The relation for the redox potential of excited electronic states is only approximate as it is based on the assumption that changes in entropy and geometry between ground and excited states are negligible.

This high oxidation potential infers that the triplet-excited duroquinone is able to oxidize CO_3^{2-}, whereby oxygen may be produced by the following sequence (Scheerer and Grätzel, 1976; Henglein and Grätzel, 1977)

$$DQ \xrightarrow{h\nu} DQ^{T}$$

$$CO_3^{2-} + DQ^{T} \rightarrow CO_3^{-} + DQ^{-}$$

$$CO_3^{-} + DQ \rightarrow CO_3 + DQ^{-}$$

$$CO_3 \rightarrow CO_2 + \tfrac{1}{2}O_2$$

$$\text{Net: } CO_3^{2-} + 2DQ \xrightarrow{h\nu} CO_2 + \tfrac{1}{2}O_2 + 2DQ^{-}$$

As the CO_2 formed regenerates CO_3^{2-} in aqueous solutions the effect of carbonate is in fact catalytic and the overall process

is that of photooxidation of water. One of the major problems in these homogeneous electron transfer reaction sequences is to prevent back reaction of the primary species. In the duroquione case, the use of micellar systems seems promising (Henglein and Grätzel, 1977; Scheerer and Grätzel, 1977) in stabilizing the charge-separated species.

The fundamental photochemical and photophysical characteristics of duroquinone have been studied by several groups (Bridge and Porter, 1958; Wilkinson et al., 1968; Hermann and Schneck, 1968; Land, 1969; Nafisi-Movaghar and Wilkinson, 1970; Bensasson and Land, 1971; Kemp and Porter, 1971; Amand and Bensasson, 1975; Amouyal and Bensasson, 1976) and parameters such as wavelength of triplet-triplet absorption, triplet-triplet absorption extinction coefficient, and intersystem crossing efficiency have been determined in several different solvents. From these studies it seems well established that the transient absorption at 490 nm with $\Delta\varepsilon_{max} = 6.95 \cdot 10^3$ $M^{-1}cm^{-1}$ (in benzene) observed by flash photolysis and pulse radiolysis is due to the lowest excited triplet state of duroquinone. The decay kinetics of triplet duroquinone have also been studied extensively and generally the decay is of mixed first- and second-order due to various non-radiative unimolecular decay channels and triplet-triplet annihilation. The question of possible self-quenching, however, still remains controversial. Land (1969) reported that triplet duroquinone in benzene is quenched by ground-state duroquinone with a rate constant of ca. $1 \cdot 10^6$ $M^{-1}s^{-1}$, while Amouyal and Bensasson (1976) were unable to observe any self-quenching in cyclohexane, ethanol, or water. These observations are probably not contradictory, as Amouyal and Bensasson (1976) investigated only a limited concentration range at low concentrations ($\leq 10^{-4}$ M) of duroquinone. From these data, however, an upper limit for the self-quenching rate constant of 10^8 $M^{-1}s^{-1}$ in cyclohexane can be inferred.

Pulse irradiation of an Ar-saturated benzene solution containing duroquinone gave a transient absorption around 490 nm. At a concentration of 10^{-2} M the absorption spectrum excited by a single electron pulse using streak camera detection was similar to the

spectrum reported by Land (1969). At $3 \cdot 10^{-2}$ M, however, some abnormalities were observed. The transient absorption spectrum at this concentration of duroquinone was somewhat broader and redshifted by ca. 10 nm (Jensen et al., 1978) compared with the spectrum at 10^{-2} M. These results are only preliminary and they should be confirmed by additional measurements before any conclusions are made. If correct, however, they might indicate that the triplet-excited duroquinone forms an excimer at high concentrations (> 10^{-2} M) of bulk duroquinone

$$DQ + DQ^T \rightleftarrows (DQ)_2^T$$

The kinetic results to be presented were obtained under the assumption that no complex formation takes place. This assumption has probably no practical importance, as the rate constants were obtained mainly from kinetic curves at low doses and/or low concentrations (< 10^{-2} M). The exception to this is the rate constant of self-quenching which is based on experimental data obtained in the concentration range 10^{-3}-10^{-1} M.

Experimental kinetic data on systems which undergo mixed first- and second-order reactions can be analysed by at least two different methods. The first, widely used in flash photolysis, is based on the differential equation for the concentration of the transient S:

$$-\frac{d(S)}{dt} = k_I(S) + 2k_{II}(S)^2$$

giving

$$-\frac{d\ln(S)}{dt} = k_I + 2k_{II}(S)$$

where the second-order reaction is supposed to consume 2 moles of S. Thus, a plot of the slopes of the logarithmic absorbance curves versus the transient absorbance should yield a straight line with intercept = k_I and slope = $2k_{II}/\Delta\varepsilon \cdot \ell$. These plots may be based either on single kinetic curves or on a series of kinetic curves obtained with increasing absorbed excitation energy, i.e. increasing initial concentration of transient. In the

latter case the individual kinetic curves are normally measured at time zero (Amouyal and Bensasson, 1975). This method has been used in a preliminary report of the present data (Jensen et al., 1978). It was, however, a poor choice for the present data, as this method hides the dose dependence of the first-order rate constants unless the plots are based on single kinetic curves. Furthermore, the uncertainty is large, as the method implies that tangential slopes of the curved logarithmic plots be determined.

The second method is based on the integrated equation for the transient absorbance:

$$\frac{A_{max}}{A} = \left(1 + \frac{2k_{II}A_{max}}{k_I \cdot \Delta\varepsilon \cdot \ell}\right) \exp(k_1 t) - \frac{2k_{II}A_{max}}{k_I \cdot \Delta\varepsilon \cdot \ell}$$

and was used above for the analysis of triplet naphthalene decay. This method will also be used in the following analysis of the triplet duroquinone data, and it should be noticed that it involves only a determination of k_I from the limiting slope at long times of the logarithmic absorbance plot, followed by a determination of the slope of the linear plot of A_{max}/A versus $\exp(k_I t)$. The latter plot is easily constructed by computer from the digitized experimental raw data. In nearly all cases the values of k_I extracted from the logarithmic absorbance plots gave good linear plots of A_{max}/A versus $\exp(k_I t)$. In a few cases the original values of k_I were changed in order to obtain linearity, but the necessary changes were always less than 20%.

All measurements of yields and time dependence were made at 500 nm. Initially this wavelength was chosen on the basis of the red-shifted absorption spectrum mentioned above. However, in view of the uncertainty of the spectra recorded at various concentrations it is still uncertain whether or not 500 nm corresponds to λ_{max}. As noted previously the analysis made here assumes $\lambda_{max} = 490$ nm. Bensasson and Land (1971) reported $\Delta\varepsilon_{490} = 6.95 \cdot 10^3\ M^{-1}cm^{-1}$ for triplet duroquinone in benzene. A value of $\Delta\varepsilon_{500}$ can be obtained by using the ratio $\Delta\varepsilon_{490}/\Delta\varepsilon_{500} = 1.40 \pm 0.04$ inferred from Land (1969) and Keene et al. (1965) giving $\Delta\varepsilon_{500} = 5.0 \cdot 10^3\ M^{-1}cm^{-1}$, which will be used in the following:

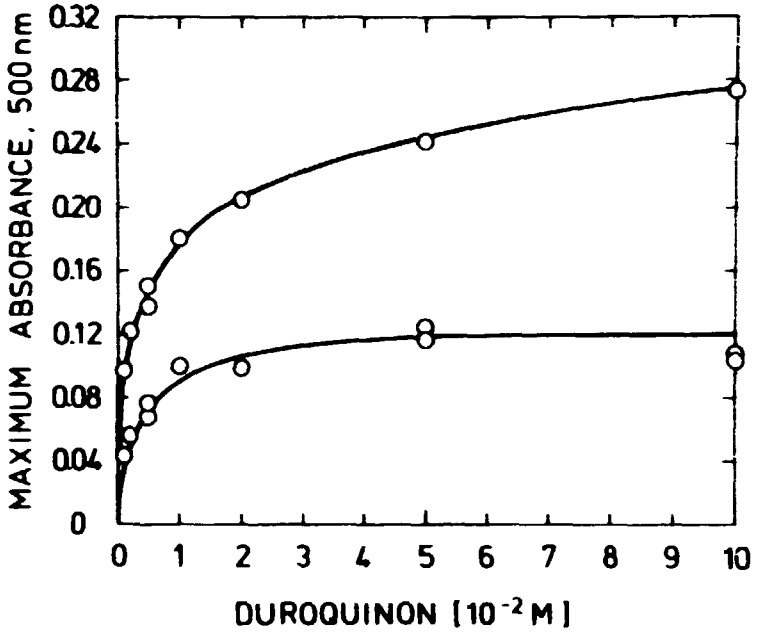


Figure 15. Transient absorption maximum at 500 nm versus concentration of ground-state bulk duroquinone. Irradiation doses were constant at 1.7 krad (lower curve) and 3.4 krad (upper curve). Optical path length = 5 cm. The curves represent the best visual fit to the experimental points.

The maximum transient absorbance at 500 nm as a function of the concentration of bulk duroquinone is shown in Fig. 15 for two different irradiation doses. From these curves a limiting value of the triplet yield at high concentration can be calculated:

$$G \cdot \Delta\varepsilon_{500} = \frac{A}{1.04 \cdot d \cdot D \cdot \ell} \cdot 10^6$$

where G is the radiation chemical yield in molecules$\cdot$(100 eV)$^{-1}$, $\Delta\varepsilon$ the difference extinction coefficient, A the transient absorbance, d the density of the medium, D the irradiation dose in krad, and ℓ the optical path length. At 10^{-1} M duroquinone concentration a value of $G \cdot \Delta\varepsilon_{500} = (1.7 \pm 0.2) \cdot 10^4$ M^{-1}cm^{-1} (100 eV)$^{-1}$ is calculated. This value is lower than the value $G \cdot \Delta\varepsilon_{500} = 2.9 \cdot 10^4$ M^{-1}cm^{-1} (100 eV)$^{-1}$ obtained from the data of Land (1969). At a concentration of $5 \cdot 10^{-3}$ M the yield $G \cdot \Delta\varepsilon_{500} = (0.93 \pm 0.01) \cdot 10^4$ M^{-1}cm^{-1} (100 eV)$^{-1}$ is calculated, which is to be compared with both the value of $1.8 \cdot 10^4$ M^{-1}cm^{-1} (100 eV)$^{-1}$ of Land (1969) and that of $1.1 \cdot 10^4$ M^{-1}cm^{-1} (100 eV)$^{-1}$ of Keene et al. (1965). Thus,

the yields obtained in this work seem to be somewhat lower than those previously reported. Probably the effective doses used for irradiation of duroquinone have been overestimated; this may be due to the making of the duroquinone irradiations with an optical cell other than the one used for irradiation dose measurements. Small differences in the thickness of the cell walls may cause significant differences in the effective doses, as the electron beam is of relatively low energy (2 MeV). Using $\varepsilon_{500} = 5 \cdot 10^3$ $M^{-1}cm^{-1}$, the value G = 3.4 $(100\ eV)^{-1}$ is obtained at a duroquinone concentration of 10^{-1} M. This value is somewhat lower than expected from the yields of triplet naphthalene in benzene, reported by Dainton et al. (1972), as G = 4.1 $(100\ eV)^{-1}$ when corrected to the extinction coefficient of $1.32 \cdot 10^4$ $M^{-1}cm^{-1}$ given by Bensasson and Land (1971). The intersystem-crossing quantum yield of naphthalene is 0.75 (Amand and Bensasson, 1975) while values of 0.94 and 1.0 have been reported for duroquinone (Nafisi-Movaghar, 1970; Amouyal and Bensasson, 1976; Amand and Bensasson, 1975). Thus, a higher radiation chemical yield of triplet duroquinone than of triplet naphthalene is to be expected as a larger proportion of the radiation-produced excited singlet states will be converted to triplet states in the case of duroquinone as compared with naphthalene. Again, the result indicates that the effective irradiation dose has been overestimated.

The decay kinetics of triplet duroquinone were analysed on the basis of the following scheme

DQ	DQ^S, $DQ^T \rightarrow DQ^T$	
DQ^T	$\rightarrow$ DQ	k_{10}
$DQ^T + DQ$	$\rightarrow$ 2DQ or other ground state products (solvent dependence)	k_{11}
$2DQ^T$	$\rightarrow$ 2DQ or other ground-state products	$2k_{12}$
$DQ^T + R\cdot$	$\rightarrow$ DQ + R$\cdot$	k_{13}

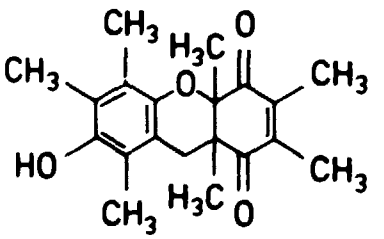
where DQ and DQ^T represent ground-state duroquinone and R$\cdot$ is a radiolytically produced radical derived from benzene. As noted above the notation $2k_{12}$ represents only the experimental second-

order rate constant for triplet-triplet annihilation. This reaction may consume two triplet-excited molecules, but it is highly probable that the sequence

$$2DQ^T \rightarrow DQ^S + DQ$$

$$DQ^S \rightarrow DQ^T \qquad \phi_{isc} \simeq 1.0$$

is part of the overall second-order depletion of triplet duroquinone. Amouyal and Bensasson (1976) assumed, in fact, that this sequence was solely responsible for the observed triplet-triplet annihilation in cyclohexane and ethanol. The proposal by these authors, however, that triplet-triplet annihilation might also be responsible for the production of diduroquinone seems unlikely, as diduroquinone has been observed as the product of preparative photochemical irradiation of duroquinone at low light intensity (Nafisi-Movaghar and Wilkinson, 1970). Diduroquinone is more likely the product of the self-quenching reaction represented by the rate constant k_{11}, at least in polar solvents.



DIDUROQUINONE

The dose dependence of the first- and second-order rate constants evaluated as noted above is shown in Figs. 16 and 17. In each case the rate constants are obtained at low concentrations of duroquinone where the self-quenching reaction makes only a minor contribution to the overall decay of triplet duroquinone. For comparison Fig. 16 also includes the rate constants extrapolated to zero duroquinone concentration, i.e. the intercepts of plots such as Fig. 18. The slopes of the two lines are

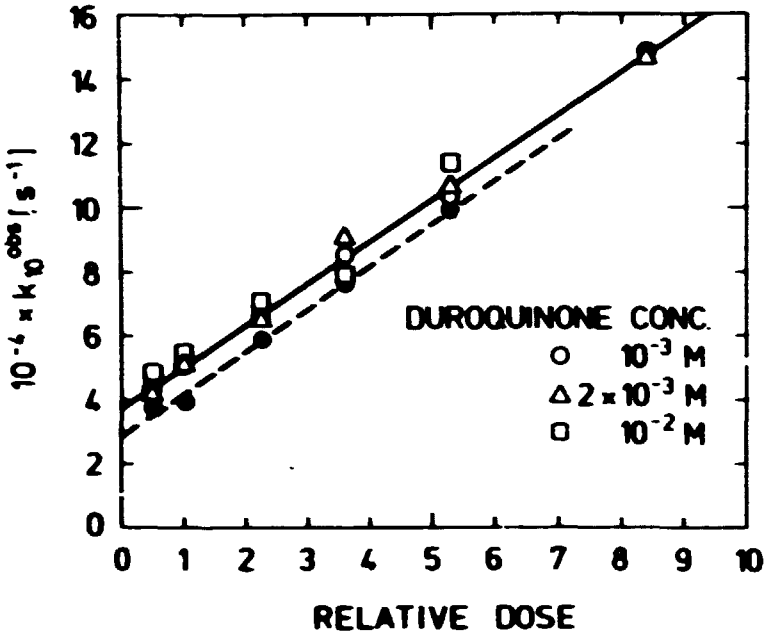


Figure 16. First-order rate constant for the decay of triplet duroquinone in benzene versus relative irradiation dose. The filled points are obtained by extrapolation to zero concentration of duroquinone.

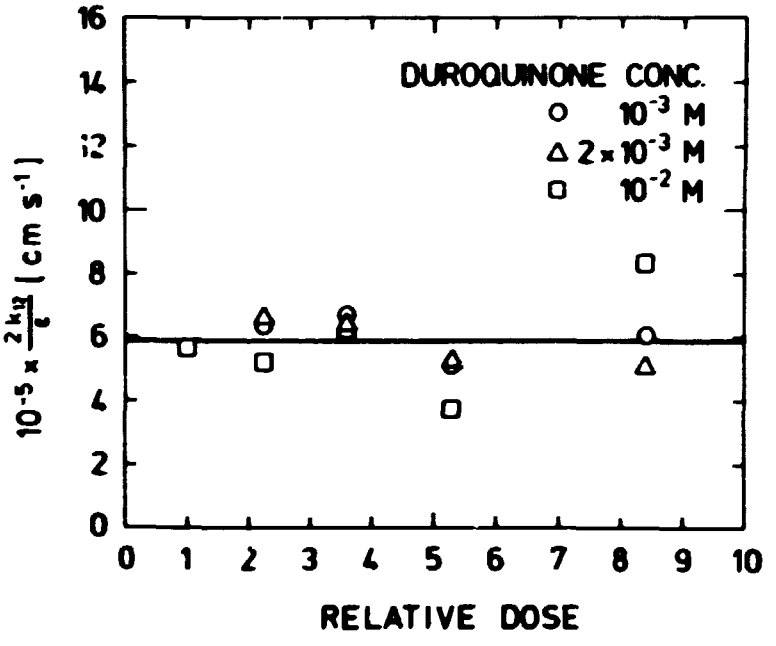


Figure 17. Second-order rate constant for the decay of triplet duroquinone in benzene versus relative irradiation dose.

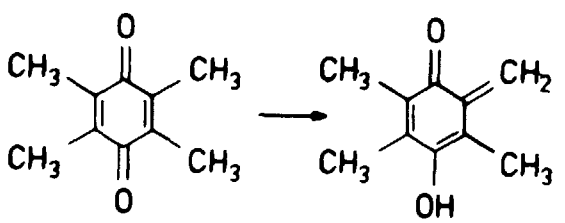
identical, and the limiting first-order rate constant at zero dose and duroquinone cencentration is $k_{10} = (2.9\pm0.2)\cdot10^4\ s^{-1}$.

The first-order rate constants increase linearly with dose and the interpretation - i.e. catalytic quenching of the excited triplet state by radiolytically produced radicals derived from the solvent - is the same as for the dose dependence of triplet naphthalene decay. From Fig. 16 a slope of $3.93\cdot10^3\ s^{-1}krad^{-1}$ can be calculated using the relation between relative and absolute doses given above. Thus, this slope is a lower limit, as the absolute doses determined by triplet naphthalene dosimetry probably are overestimated in relation to the measurements on duroquinone. Using the G-value of free radicals in irradiated benzene given above, we obtain $k_{13} \geq 5.5\cdot10^9\ M^{-1}s^{-1}$ for the bimolecular rate constant of the catalytic quenching process; this is to be compared with the spin-corrected diffusion-controlled rate constant of $3.7\cdot10^9\ M^{-1}s^{-1}$ for a reaction between two species of equal size.

As observed for naphthalene the second-order rate constant of triplet decay is independent of dose, the mean value being $2k_{12}/\Delta\varepsilon_{500} = (5.9\pm1.1)\cdot10^5\ cm\ s^{-1}$ or $2k_{12} = (3.0\pm0.6)\cdot10^9\ M^{-1}s^{-1}$. This rate constant has not been determined by flash photolysis in benzene, but Amouyal and Bensasson (1976) reported a value of $4.2\cdot10^9\ M^{-1}s^{-1}$ in cyclohexane. Taking the lower viscosity of benzene compared with cyclohexane (0.65 cp and 1.02 cp respectively) into account, it is clear, however, that the rate constant is significantly lower in benzene than in cyclohexane. The lower rate constant in benzene can hardly be explained by differences in the type of processes responsible for the triplet-triplet annihilation in benzene and cyclohexane. Rather, the low rate constant in benzene might be due to a charge transfer interaction between triplet-excited duroquinone and benzene. Such an interaction could slow down the diffusion of the triplet duroquinone molecules or prevent the close approach of two triplet-excited molecules necessary for effective spin exchange. The possibility of a charge transfer interaction between triplet duroquinone and benzene was first pointed out by Land (1969) on the basis of the optical spectra in cyclohexane and benzene, but the phenomenon has not been studied since.

The observed first-order rate constants increase linearly with the concentration of bulk duroquinone at constant dose, as shown in Fig. 18. The concentration dependence was evaluated at five different doses in the range 1.7-18 krad. The mean value of the slopes is $k_{11} = (1.20 \pm 0.25) \cdot 10^6\ M^{-1}s^{-1}$, which is the bimolecular rate constant for the self-quenching reaction. The intercepts of these plots give the first-order rate constants at zero duroquinone concentration but at increasing irradiation dose. These values are included in Fig. 16 above. The increased rate of decay at high concentrations of duroquinone could be due to an impurity (e.g. durohydroquinone), which quenches the excited triplet state. However, this possibility is unlikely because 1) the duroquinone used was carefully purified, 2) the observed first-order rate constants agree with the values reported by Land (1969) and the limiting value k_{10} is a factor of 3 lower than the first-order rate constant obtained by Kemp and Porter (1971) using flash photolysis, and 3) the value of k_{11} agrees with the approximate value $1 \cdot 10^6\ M^{-1}s^{-1}$ reported by Land (1969).

The increased rate of decay at high concentrations of duroquinone is due instead to self-quenching processes, the nature of these being dependent on the polarity of the solvent. In polar solvents the triplet state may isomerize to the o-quinone-methid from



which diduroquinone may be formed by a cyclic addition reaction to ground-state duroquinone (Kemp and Porter, 1971). In apolar solvents such as benzene used here, the self-quenching process is more likely to be physical quenching of the triplet-excited duroquinone by ground-state duroquinone, as no diduroquinone was observed by preparative photochemical irradiation of 10^{-2} M duroquinone in benzene (Nafisi-Movaghar and Wilkinson, 1970).

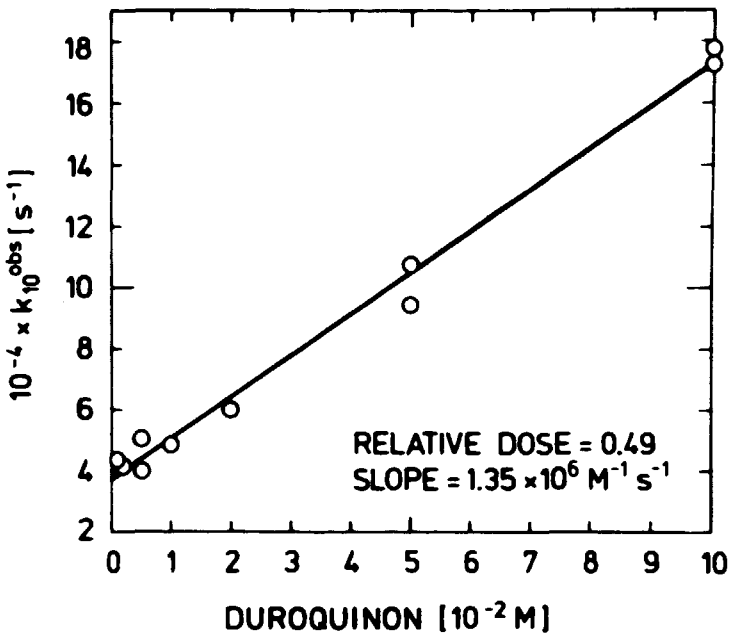


Figure 18. First-order rate constant for the decay of triplet duroquinone in benzene versus concentration of ground-state bulk duroquinone. The relative irradiation dose was constant 0.49 (1.7 krad).

3.2.5. Summary and comparative remarks

The results on triplet-excited states obtained in this work are summarized in Table III along with some data from the literature. Most of the data reported have been discussed extensively above. Here the discussion will be focused on the rate parameters of chlorophyll a and β-carotene and on the rate constants derived from the dose dependencies of the first-order rate constants.

Table III. Summary of photophysical parameters.

	E_S kJ/mol	E_T kJ/mol	λ_{max}^{TT} nm	$\Delta\epsilon_{max}^{TT}$ 10^4 $M^{-1}cm^{-1}$	$k(T \rightarrow S_o)$ 10^4 s^{-1}	$2k(T \rightarrow T)$ 10^9 $M^{-1}s^{-1}$	$k(M^T \rightarrow S_o)$ 10^9 $M^{-1}s^{-1}$	$k(T \rightarrow R\cdot)$ 10^9 $M^{-1}s^{-1}$	$k(T \rightarrow S_o)$ 10^6 $M^{-1}s^{-1}$
Naphthalene	380	254	400 420	1.32	4.1±0.3	6.1±0.9	-	3.7±0.7	-
Chlorophyll a	181	128	470	3.4±0.3	2.2±0.3	1.4±0.3	3.6±0.6	< 1	< 50
β-Carotene	248	88-105	530	23.8	29±3	3.6±0.4	10.7±1.2	< 1	-
Duroquinone	335	231	490	0.695	2.9±0.2	3.0±0.6	-	≥ 5.5±0.6	1.2±0.3

The second-order rate constant for decay of triplet chlorophyll is in reasonable agreement with the value $2k = 2.1 \cdot 10^9$ $M^{-1}s^{-1}$ reported by Linschitz and Sarkanen (1958). Also the rate constant for triplet energy transfer from naphthalene to β-carotene compares well with the value $1.8 \cdot 10^{10}$ $M^{-1}s^{-1}$ reported by Bensasson et al. (1977) for energy transfer from biphenyl to β-carotene in hexane, as both values are near the diffusion-limited level, $k_D = 8RT/3000\eta$, of $1.1 \cdot 10^{10}$ $M^{-1}s^{-1}$ and $2.2 \cdot 10^{10}$ $M^{-1}s^{-1}$ for benzene and hexane, respectively. Values of the second-order rate constant for the decay of triplet β-carotene and the rate constant for triplet energy transfer to chlorophyll *a* from naphthalene have not been reported previously. Comparing the bimolecular rate constants of chlorophyll *a* and β-carotene one notices that triplet-triplet annihilation as well as the triplet energy transfer process seems to be 2.5-3 times less efficient for chlorophyll *a* than for β-carotene. This difference may be due to steric hindrance when chlorophyll *a* is involved, impeding the proper relative orientation of the molecules in the encounter complexes. Both types of processes studied can proceed only if there is a large degree of electronic interaction among the reacting molecules (Lamola, 1969). For chlorophyll *a* this is possible only if the molecules have a coplanar orientation in the encounter complex, whereas the rod-shaped β-carotene molecule has a larger number of favourable orientations, where close contact with another β-carotene or naphthalene molecule is possible.

The mechanism of the triplet-triplet annihilation processes of chlorophyll *a* and β-carotene cannot be established from the present data. However, the rate constants are lower than the diffusion-limited rate constant $2k_D = 1.1 \cdot 10^{10}$ $M^{-1}s^{-1}$ for a bimolecular reaction in benzene consuming two identical species ($k_D = 4RT/3000\eta$). Parker and Joyce (1967) have reported on the yields of delayed fluorescence of chlorophyll *a* and *b* in ethanol. From these measurements they obtained values of the efficiency of singlet state production in the annihilation process. These values were based on an assumed diffusion-controlled annihilation process. Although the results are questionable as they imply intersystem crossing efficiencies of the chlorophylls

which are 2-3 times lower than expected, corrections can be made for the lower experimental rate constants. In this way a singlet formation efficiency of $0.11 < p_e < 0.76$ is obtained, where the singlet formation, p_e, is defined as above in the treatment of triplet naphthalene. This derivation is based on the intersystem crossing efficiency of 0.64 determined by Bowers and Porter (1967) for chlorophyll a in ether and on the assumption that the singlet formation efficiency is the same in ethanol and benzene. The singlet formation efficiency is thus rather badly determined, and furthermore the upper limit is clearly too high, as it should be lower than 0.5 because of the energetic restrictions.

For β-carotene no reports on the triplet-triplet annihilation process exist. As shown in Table III, formation of the ^1B_u state is impossible as the triplet energy is lower than half the energy of the ^1B_u state. Recently, however, Thrash et al. (1977) provided evidence for locating the optically forbidden ^1A_g state at least 42 kJ mole^{-1} below the ^1B_u state. This makes the production of the ^1A_g state in the triplet-triplet annihilation process energetically feasible, and in view of the relatively efficient triplet-triplet annihilation process for β-carotene it is proposed that the process

$$2\ \beta\text{-carotene}\ (^3B_u)^* \rightarrow \beta\text{-carotene}\ (^1A_g)^* + \beta\text{-carotene}\ (^1A_g)$$

is a major channel in the second-order deactivation of triplet β-carotene. The question of whether or not this process is operating in photosynthesis calls for careful experimental studies of triplet states in biological systems, but here it should be noted that β-carotene in its excited ^1A_g state may be an ideal donor for transfer of singlet energy to the chlorophylls (Thrash et al., 1979). Thus the triplet-triplet annihilation reaction producing the excited ^1A_g state could be part of a reaction sequence *in vivo* whereby wasted triplet energy is channelled back to chlorophyll a or b. Although this process involves the interaction of two excited molecules, it might be effective due to the long lifetime of triplet β-carotene (ca. 5 μs) in biological systems (Witt, 1971; Breton et al., 1979).

Considering the rate constants for quenching of the excited triplet states by the radiolytically produced radicals, one notices that the compounds studied may be divided into two groups, (i) chlorophyll a and β-carotene for which the first-order rate constants are apparently independent (or nearly so) of irradiation dose, and (ii) naphthalene and duroquinone for which a clear dose dependence was observed. Naphthalene and duroquinone were both acting as the primary acceptors of the triplet excitation produced in the solvent molecules while both chlorophyll a and β-carotene were sensitized by triplet naphthalene. However, it seems unlikely that these differences in the modes of production of the triplet states are responsible for the differences in the observed dose dependencies, as the typical times involved were almost the same for triplet duroquinone and triplet β-carotene. Thus, approximately the same amount of radiolytically produced free radicals must have been present in the two systems, if it is assumed that the free radicals disappear by e.g. recombination independently of the excited states.

Another striking difference between the compounds in the two groups appears when comparing the sizes of their conjugated electronic systems; naphthalene and duroquinone are small compared with chlorophyll a and β-carotene. Therefore, it is to be expected that the unpaired spin density is much more localized in the triplet excited states of duroquinone and naphthalene compared with that of chlorophyll a and β-carotene. This difference in the spin densities may in turn relatively enhance the interaction between the triplet states of duroquinone/naphthalene and the quenching free radicals, thereby increasing the probability of spin exchange, i.e. increasing the apparent quenching rate constants of the small triplet molecules compared with that of the large triplet molecules. This explanation can be only tentative, but it should be noticed that the slight difference in the rate constants between duroquinone and naphthalene is in accordance with the interpretation given, as the spin density of triplet naphthalene should be more delocalized than that of triplet duroquinone.

3.3. Time-resolved resonance Raman spectroscopy

During the last decade resonance Raman spectroscopy has proven to be a powerful technique in studies of biological systems and unstable intermediates (Carey, 1978; Hester, 1978). These studies have taken advantage of two important characteristics of resonance Raman spectroscopy, namely, the possibility of obtaining vibrational spectra of aqueous systems, which also applies to normal Raman spectroscopy, and the high sensitivity due to the resonance enhancement (Johnson and Peticolas, 1976; Spiro and Stein, 1977). For large biological molecules it has furthermore been valuable that only vibrational modes coupled to the chromophore which is excited are resonance enhanced. Thus, the number of detected bands is reduced as compared with the number of bands observed in, e.g., a normal Raman spectrum of the same system. The reduced number of detected Raman bands under these circumstances will, of course, preclude a complete vibrational analysis of the actual system. This objection is, however, of little value when biological systems are studied, as in most cases the aims of such studies are to elucidate stationary structural features or chemically induced structural changes of specific sites rather than a vibrational analysis of the system as a whole. If the system of interest does not contain a chromophore absorbing in the accessible spectral range (e.g., proteins) artificial chromophores, so-called resonance Raman labels, may in some cases be introduced into the molecule (Carey and Schneider, 1978).

The high sensitivity of resonance Raman spectroscopy also allows spectra to be recorded in short time intervals using pulsed lasers as excitation source and electro-optical multichannel detectors for collecting the signals. This technique was pioneered by Bridoux and Delhaye (1976) and was subsequently used by Pagsberg and coworkers to obtain resonance Raman spectra of the stable free radical DPPH in dilute solution (Wilbrandt et al., 1975), and the transient radical anion of p-terphenyl produced by pulse radiolysis (Pagsberg et al., 1976). This work initiated several experiments at various groups and the combination of pulsed lasers and multichannel spectrometers is now used in

several types of resonance Raman experiments on stable as well as transient species. The potentials of these techniques seem unlimited, as the time-resolution may be expanded to cover the picosecond range (Dallinger et al., 1979). Thus, flash photolysis and pulse radiolysis may now yield transient vibrational spectra, which contain much more information about the transient species than does the normally recorded visible and UV absorption spectra. Currently, however, only 2-3 groups, including the Risø group, are using time-resolved resonance Raman spectroscopy in conjunction with pulse radiolysis and flash photolysis.

In relation to photosynthesis, conventional resonance Raman spectroscopy has been used to study the properties of the chlorophylls (Lutz, 1977; Lutz et al., 1979) and carotenoids (Lutz et al., 1978; Agalidis et al., 1980) in photosynthetic systems. The detailed information resulting from these studies makes it reasonable to expect that time-resolved resonance Raman studies of transients (radical ions and excited states) involving photosynthetic pigments in vitro and in biological preparations might yield important information on the identity and function of these transients in photosynthesis. In the sole attempts in this direction with regard to the chlorophylls, use has been made of conventional resonance Raman spectroscopy in studies of bacteriochlorophyll and its radical cation both in solution and in biological preparations (Cotton and Van Duyne, 1978; Lutz and Kleo, 1979). Conventional techniques could be used in these studies as the radical cations of the chlorophylls are remarkably stable.

Apart from the spectroscopic data on the radical cations, these studies of the chlorophyll radical cations can hardly add new knowledge concerning the existence of these species as transients in photosynthesis of green plants and bacteria. The radical cations have been well characterized by ESR, endor, and optical absorption spectroscopy, and there is close correspondence between in vivo and in vitro results.

The picture is more obscure with regard to the identity of the primary acceptors, especially in the photosynthesis of higher green plants (see, e.g., Fujita et al., 1978) where the infor-

mation obtained from ESR is far less conclusive presumably due to the interaction between the reduced acceptor and nearby Fe^{2+}. The results on the radical cation of bacteriochlorophyll have demonstrated that the changes in vibrational frequencies upon removal of one electron are sufficient for identification of the radical cation in vivo. Consequently, resonance Raman spectroscopy should also be able to provide positive evidence concerning the radical anions in biological systems as the frequency changes that occur upon electron attachment are expected to be of the same magnitude. Moreover, resonance Raman spectroscopy would be the method of choice to distinguish between chlorophylls and pheophytins as electron acceptors because the chlorophylls have characteristic low-frequency bands related to the vibrational modes involving magnesium (Lutz, 1974; 1977).

Work related to transients involving carotenoids would naturally be concentrated on the excited triplet states which have been identified by absorption spectroscopy in photosynthetic systems. Time-resolved resonance Raman spectroscopy should be able to confirm this assignment conclusively and might eventually give a clue to the problem concerning the apparent existence of reaction centre bound cis-carotenoids as compared with the bulk all-trans carotenoids in photosynthetic bacteria (Lutz et al., 1978; Agalidis et al., 1980).

3.3.1. Results

One of the original goals of the present project was to obtain the resonance Raman spectrum of the chlorophyll a radical anion in solution. This task has not, however, been accomplished yet. Further experiments will be conducted later with a new laser of a narrower spectral band width.

Experiments on the triplet states of duroquinone and chlorophyll a have also been unsuccessful. In the duroquinone case no Raman bands, transient or stationary, were observed even at duroquinone concentrations as high as $5 \cdot 10^{-2}$ M and at the maximum irradiation dose. The laser wavelength was tuned to 495.0 nm in these experiments. The absence of bands from ground-state duro-

quinone suggests that the scattering efficiency is very low, and it does not leave much hope for observing Raman bands from the triplet state.

In the case of triplet chlorophyll *a* weak bands from the ground state were observed using $3.8 \cdot 10^{-4}$ M chlorophyll *a* in benzene containing 10^{-2} M naphthalene. On pulse irradiation with maximum dose extremely weak transient bands could be observed, but the spectra with and without irradiation were all of very poor quality. This can easily be explained, however, by the low concentration of chlorophyll *a* used and the low spectral resolution of the combined experimental set-up. The laser wavelength in these experiments was 469.9 nm. In this case the experimental conditions can be improved by using larger amounts of chlorophyll *a* and a laser with a narrower spectral band width. The spectral resolution seems to be very critical when chlorophylls are studied, as the Raman spectra contain a large number of nearby bands which merge into a continuum at low spectral resolution.

In contrast to these experiments the results obtained on the triplet state of all-trans-ß-carotene are very informative. These results are described in the submitted manuscript in the appendix to which the reader is referred.

4. RADICAL ANION OF CHLOROPHYLL *a*

4.1. Introduction

The idea that radical anions of the chlorophylls might be involved in the primary photochemical processes of photosynthesis is an old one (see e.g. Kamen, 1963), but only recently has experimental evidence in support of this idea appeared. The first results were obtained on bacterial systems by picosecond flash photolysis; they indicated that bacteriopheophytin may be the primary electron acceptor in the bacteria Rhodopseudomonas

sphaeroides (Rockley et al., 1975; Fajer et al., 1975; Kaufmann et al., 1975). Subsequently, a large amount of data on different bacterial systems have been presented. These results on bacterial photosynthesis could be taken as a strong indication that similar reactions were operating in higher green plants. The experimental difficulties in studying the primary reactions of green plant photosynthesis are, however, formidable in that well reaction centres related to the two photosystems, PSI and PSII, have not been isolated. Hence, a differentiation between optical changes due to the two photosystems is difficult. Following the reports by Klimov et al. (1977) on pheophytin a as primary acceptor in PSII and by Shuvalov et al. (1979) on chlorophyll a as primary acceptor in PSI, interlinked by the conclusive optical identification of the radical anions of chlorophyll a and pheophytin a (Fujita et al., 1978), a coherent picture of the primary charge-separating photochemical reactions of photosynthesis begins to emerge (see also Sauer, 1979 for a recent review).

As mentioned previously, the original aim of this project was to study the radical ions of chlorophylls, especially the radical anion of chlorophyll a. The latest developments in the field have proved that this aim was in fact "hot matter". This report will describe the spectral and kinetic results obtained. Some of the results have been discussed in preliminary form elsewhere (Jensen, 1977). Hence, the conclusions presented here differ in some cases from those previously given. The optical absorption spectra to be presented were all obtained by pulse radiolysis of chlorophyll a in solution. Preparation of stable solutions of the radical anion of chlorophyll a by various chemical and photochemical methods was attempted, but unsuccessfully. In all cases various degradation products were obtained, and as all the products could be assigned to previously described reactions no

4.2. Results and discussion

4.2.1. Chlorophyll a in tetrahydrofuran

Pulse irradiation of $1.96 \cdot 10^{-5}$ M chlorophyll a in Ar-saturated tetrahydrofuran (THF) gave a transient absorption with λ_{max} = 455 nm as shown in Fig. 19. The full absorption was developed immediately after the 30-ns irradiation pulse. The initial spec-

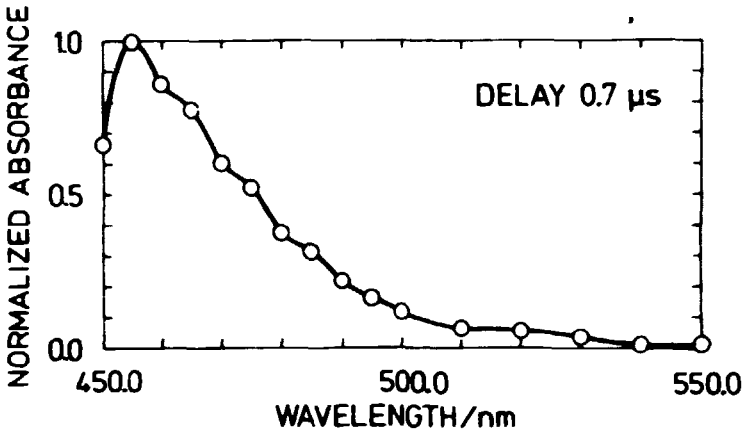


Figure 19. Normalized transient difference absorption spectrum after irradiation of $19.6 \cdot 10^{-6}$ M chlorophyll a in pure THF. The irradiation dose was ca. 18 krad. The spectrum is normalized against A = 0.117. Optical path length = 2 cm.

trum relaxes with a dose dependent half-life into a broad spectrum as shown in Fig. 20. This initial decay has a half-life of 5-40 µs, but further decay was observed on longer time scales (milliseconds). Only the initial decay was analysed. The dose dependence indicated an apparent second-order reaction and this was verified by the linear plots of $\tau_{\frac{1}{2}}$ versus $1/A_{max}$ and $(A_{max}-A_{\infty})/(A-A_{\infty})$ versus time. The reaction responsible for this decay is probably that between the chlorophyll a radical anion and the solvent cation produced in the pulse:

$$Chl^{\bullet -} + C_4H_8O^{\bullet +} \rightarrow \text{or} \begin{matrix} Chl + C_4H_8O \\ ChlH^{\bullet} + C_4H_7O^{\bullet} \end{matrix} \qquad k_{14}$$

(alternatively: $Chl^{\bullet -} + H^{+}_{solv} \rightarrow ChlH^{\bullet}$)

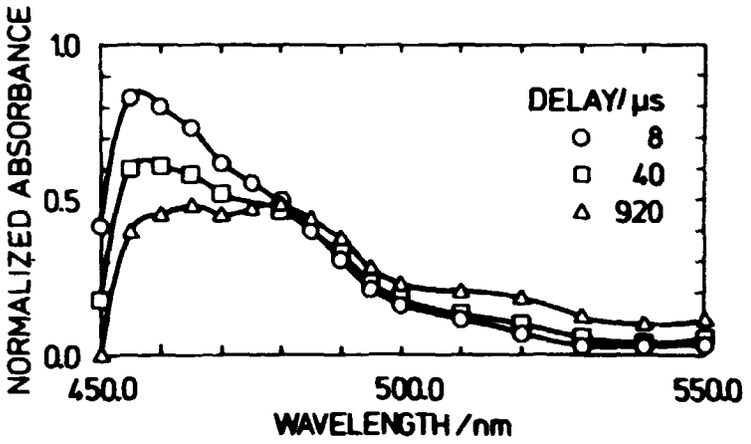


Figure 20. Normalized transient difference absorption spectra at various delays after irradiation of $19.6 \cdot 10^{-6}$ M chlorophyll a in pure THF. The irradiation dose was ca. 18 krad. The spectra are normalized against A = 0.092. Optical path length = 2 cm.

and from the second-order plots we determine $k_{14}/\Delta\varepsilon_{460} = (2.9 \pm 0.3) \cdot 10^{6}$ cm s^{-1}. Using $\Delta\varepsilon_{460} = 1.6 \cdot 10^{4}$ $M^{-1}cm^{-1}$ inferred from Fujita et al. (1978) we obtain $k_{14} = (4.6 \pm 0.5) \cdot 10^{10}$ $M^{-1}s^{-1}$.

At a constant concentration of chlorophyll a the maximum transient absorbance at the end of the pulse increased with increasing irradiation dose, but the relation was not linear. Instead the maximum transient absorbance levelled off at high irradiation doses reaching an asymptotic value at the highest doses. Previously (Jensen, 1977), this behaviour was interpreted as an indication of full conversion of chlorophyll a to its radical anion. However, it is more likely due to competition between the reactions of the solvated electrons with chlorophyll a and solvent cations, respectively. The latter process is apparently of second order and its relative importance will increase with increasing irradiation dose. Using the rate constant $k_{15} = 2 \cdot 10^{12}$ $M^{-1}s^{-1}$ for the reaction

$$e^-_{solv} + C_4H_8O^{+\bullet} \rightarrow \text{Products} \qquad k_{15}$$

and the value $G(e^-_{solv}) = 0.39$ $(100\ eV)^{-1}$, both determined by Jou and Dorfman (1973), along with an estimated rate constant for

$$C_4H_8O^{+\cdot} + C_4H_8O \rightarrow \text{Products} \qquad k_{16}$$

of $k_{16} = 1.2 \cdot 10^{12}\ M^{-1}s^{-1}$, it was possible to estimate that $k_{17} \geq 2 \cdot 10^{11}\ M^{-1}s^{-1}$ for the reaction (in THF)

$$e^-_{solv} + Chl \rightarrow Chl^{-\cdot} \qquad k_{17}$$

by computer simulation of the yields (end of pulse) of chlorophyll a radical anion. The rate constant k_{16} was obtained from a study of the reaction between dimethylether and its corresponding molecular cation (Gupta et al., 1967). It is not possible to obtain more than a rough estimate of the rate constant k_{17} as the calculations involved simulation of the system during the electron pulse where inhomogeneous kinetics prevail. The estimated rate constant is, however, in agreement with the rate constant for the reaction between solvated electrons and pyrene in THF, $k = 1.2 \cdot 10^{11}\ M^{-1}s^{-1}$, reported by Jou and Dorfman (1973). These large bimolecular rate constants reflect the relatively high mobility of the free electrons in THF (Dodelet and Freeman, 1975).

The addition of 2% (v/v) triethylamine (TEA) to the system causes no observable changes in the optical absorption of the ground state chlorophyll a. Thus, TEA apparently has no influence on the solvation of chlorophyll a. Upon irradiation, however, dramatic changes are observed. The initial end-of-pulse spectrum is identical to the spectrum observed in pure THF, but in the presence of TEA an increase in absorption is found within 10 μs after the electron pulse, as shown in Fig. 21. The species responsible for this additional absorption (λ_{max} = 495 nm) is apparently different from the initially formed transient. At longer times the presence of TEA has a strong influence on both the effective half-life, which is ca. 50 times longer than in pure THF, and on the product distribution as shown in Fig. 22. Here one notices the relatively higher absorbance at 510-530 nm in the final spectrum as compared with the final spectrum in the absence of TEA (Fig. 20). The decay was of complex order, and no determination of rate constants was attempted. The overall kinetics, however, clearly indicated that the initially

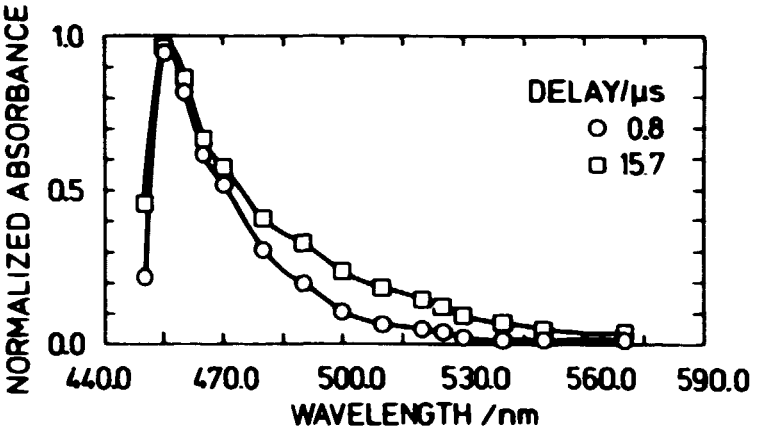


Figure 21. Normalized transient difference absorption spectra at various delays after irradiation of $1.20 \cdot 10^{-4}$ M chlorophyll a in THF containing 2% TEA. The irradiation dose was ca. 18 krad. The spectra are normalized against A = 0.490. Optical path length = 2 cm.

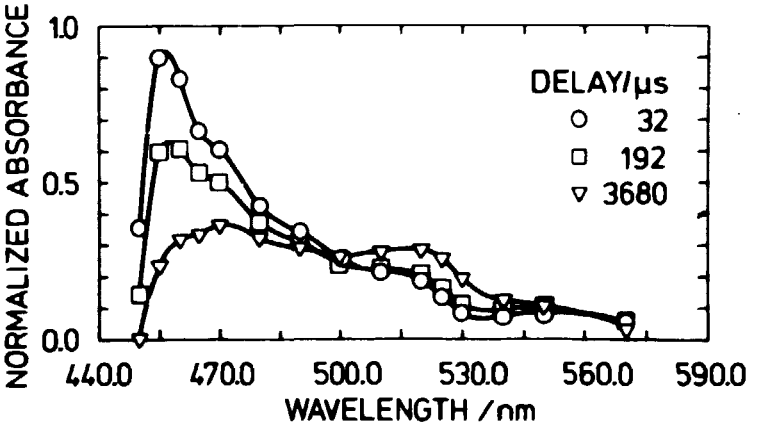


Figure 22. Normalized transient difference absorption spectra at various delays after irradiation of $1.20 \cdot 10^{-4}$ M chlorophyll a in THF containing 2% TEA. The irradiation dose was ca. 18 krad. The spectra are normalized against A = 0.460. Optical path length = 2 cm.

formed transient with λ_{max} = 455 nm is precursor to the transient with λ_{max} = 520 nm, as illustrated in Fig. 23.

The initially formed transient in pure THF and THF + TEA with λ_{max} = 455 nm can safely be assigned to the radical anion of chlorophyll a, as the difference spectra are in agreement with

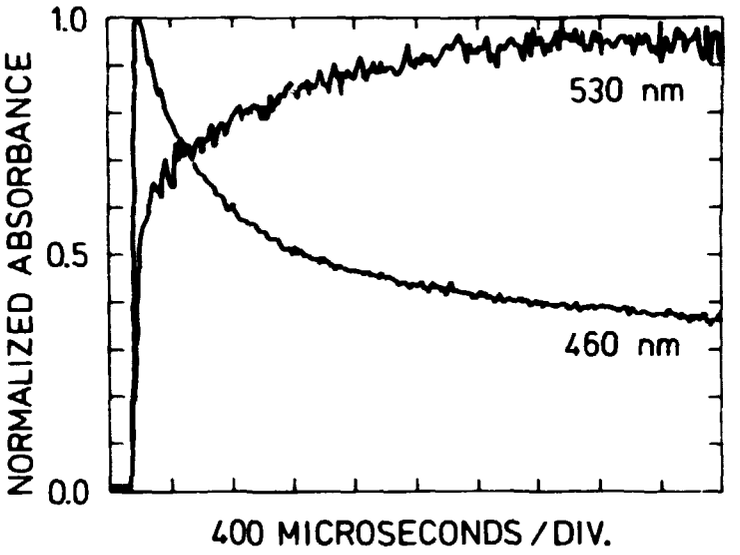


Figure 23. Kinetic curves measured at 460 nm (decay) and 530 nm (formation) after irradiation of $2.50 \cdot 10^{-5}$ M chlorophyll a in THF containing 2% TEA. The irradiation dose was ca. 18 krad.

the spectrum reported by Fujita et al. (1978) on the basis of electrochemical reduction of chlorophyll a in dimethylformamide. Moreover, the increased lifetime in the presence of TEA supports this assignment, as the presence of a strong base (e.g. TEA) would be expected to delay the protonation of the radical anion. Unfortunately, the dependence upon base strength cannot be studied because only a very limited number of bases can be used. This is due to the susceptibility of chlorophyll a against attack by strong bases such as hydroxide ion, alkoxide ions, and primary and secondary amines, which all cleaves the cyclopentanone ring of chlorophyll a (Seely, 1966). A study of the dependence of the lifetime upon the concentration of TEA has not been attempted.

The overall decay of the transient absorption is most likely caused by a variety of different reactions such as reoxidation, protonation and H-abstraction by radiolytically produced free radicals derived from THF. Furthermore, various reactions between bulk chlorophyll a and the free radicals are to be expected. All reactions involving free radicals produced radio-

lytically from THF should be apparent first-order processes, as the yield of free radicals by irradiation of THF is as high as $G = 6.0\ (100\ eV)^{-1}$ (see Salmon et al., 1974). Hence, the major reaction in pure THF seems to be protonation eventually mixed with reoxidation because these reactions are both in agreement with the observed second-order decay.

The transient product absorbing at 520 nm has previously been assigned to the so-called "Krasnovsky pigment" (Krasnovsky, 1960; Scheer and Katz, 1974), i.e. a doubly reduced chlorophyll _a_ which has been shown to be β,δ-dihydrochlorophyll _a_ (Scheer and Katz, 1974). If this assignment is correct then the product is most likely produced by the sequence

$$Chl^{-\bullet} + H^{+}_{solv} \rightarrow Chl{\bullet}H{\bullet}$$

$$ChlH{\bullet} + Chl^{-\bullet} \rightarrow ChlH^{-} + Chl$$

$$ChlH^{-} + H^{+}_{solv} \rightarrow ChlH_2$$

Disproportionation of the chlorophyll _a_ radical anion producing the dianion can scarcely explain the observed rate of formation of the 520 nm product because the disproportionation constant is extremely low (Jensen, 1977). The previous suggestion (Jensen, 1977) that the presence of TEA might change the relative reduction potentials for formation of anion and dianion, thereby increasing the disproportionation constant, cannot explain the results. Cyclic voltammetry of chlorophyll _a_ in THF containing tetrabutylammonium tetrafluoroborate as supporting electrolyte showed that $\Delta E = E^{2}_{\frac{1}{2}} - E^{1}_{\frac{1}{2}}$, where $E^{1}_{\frac{1}{2}}$ and $E^{2}_{\frac{1}{2}}$ represent the reversible reduction potentials of chlorophyll _a_, is nearly independent of the concentration of TEA up to > 10% (v/v) of added TEA.

The transient product at 520 nm may, however, also be due to the so-called "phase test intermediate" (Weller, 1954; Felton et al., 1964; Seely, 1966), which is the closed shell anion produced by deprotonation of chlorophyll _a_ at the cyclopentanone ring. The same product may be produced by addition of an electron to chlorophyll _a_, followed by H-atom abstraction from the

cyclopentanone ring. Abstraction of an H-atom could easily be explained by the presence of the above-mentioned free radicals derived from THF.

4.2.2. Chlorophyll *a* in 2-propanol

Pulse irradiation of $2.5 \cdot 10^{-5}$ M chlorophyll *a* in Ar-saturated 2-propanol with a dose of 5.6 krad gave a transient absorption with a complex kinetic picture. Initially, a short-lived absorption ($\tau_{\frac{1}{2}} \approx 500$ ns) was observed at all wavelengths. This transient absorption decayed to a plateau followed by an increase in absorption over 400 μs. On longer time scales the absorption again decayed with $\tau_{\frac{1}{2}} \approx 3$ ms at 470 nm to a final absorption, which was stable for longer than 40 ms. The initial short-lived absorption, which was fully developed immediately after the electron pulse, can be assigned to the solvated electrons produced by ionization of the solvent. The initial absorption increased gradually towards longer wavelengths and no structure was observed in this absorption, in agreement with the reported absorption spectrum of solvated electrons in 2-propanol (Sauer et al., 1965). The transient absorption spectra recorded 9.5 μs (i.e. after decay of the solvated electrons) and 385 μs after the electron pulse are shown in Fig. 24 along with kinetic curves measured at λ = 465 nm, 490 nm, and 550 nm. The spectrum obtained 9.5 μs after the electron pulse has $\lambda_{max} = 455$ nm. Furthermore, it should be stressed that the apparent fine structure around 460 nm in the 385-μs spectrum is real. The initial absorbance and the 9.5-μs absorbance measured at 470 nm both increased linearly with irradiation dose up to at least 20 krad. The absolute doses were determined from the absorption of the solvated electrons using $G \cdot \Delta\varepsilon_{470} = 5.05 \cdot 10^{3}$ $M^{-1}cm^{-1}$ $(100\ eV)^{-1}$ inferred from Sauer et al. (1965).

The transient spectrum recorded with 9.5 μs delay can be assigned to the radical anion of chlorophyll *a*, as the full spectrum is in complete agreement with that reported by Fujita et al. (1978). Having established this assignment, the rate constant for the reaction

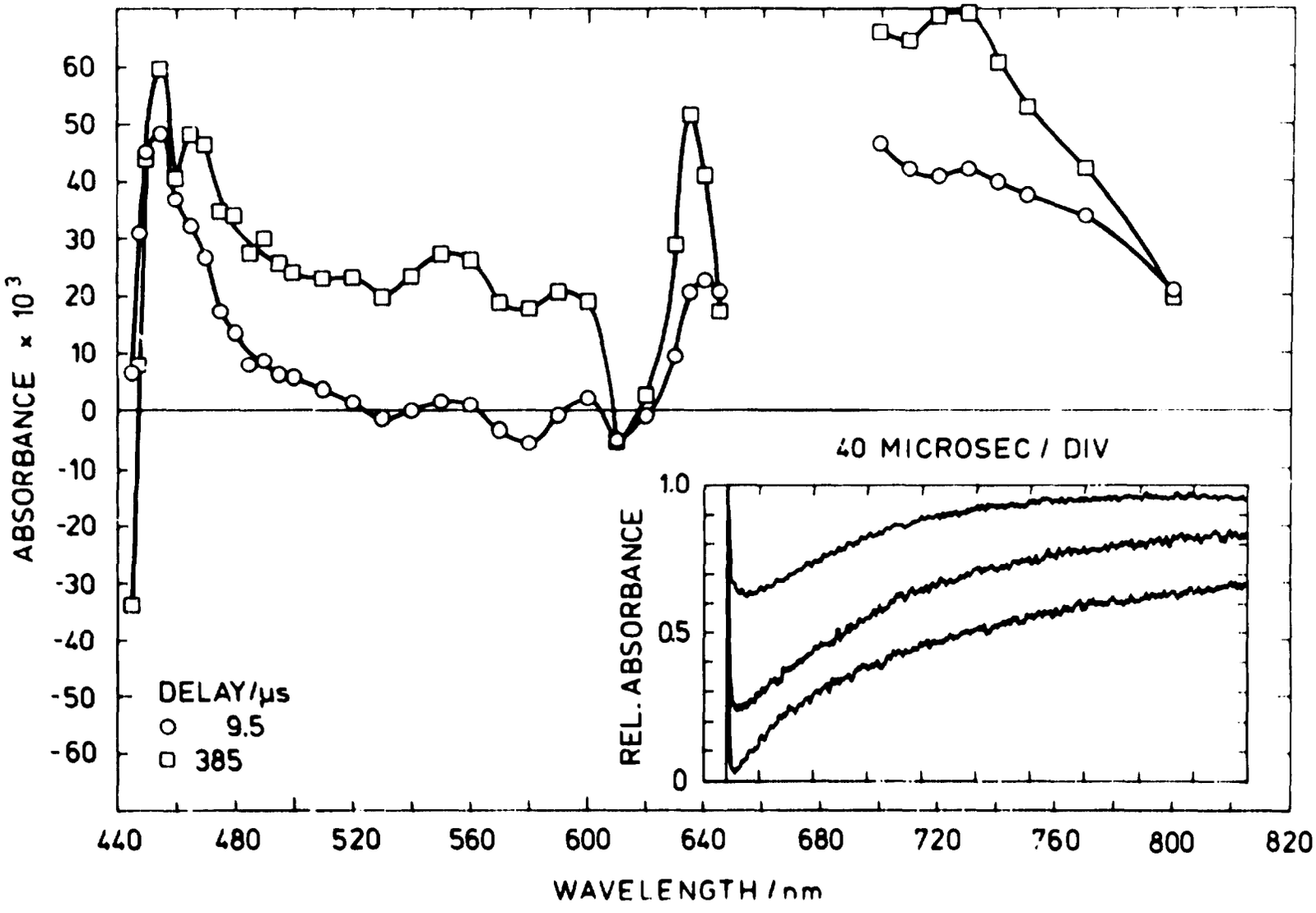


Figure 24. Transient difference absorption spectra at various delays after irradiation of $2.50 \cdot 10^{-5}$ M chlorophyll a in pure 2-propanol. The irradiation dose was ca. 5.6 krad. Optical path length 2 cm. Insert: Kinetic curves under the same conditions measured at 465 nm (upper), 490 nm (middle), and 550 nm (lower).

$$e^-_{solv} + Chla \rightarrow Chla^{-\bullet} \qquad k_{18}$$

can be calculated from the observed half-life of the solvated electrons and the observed yields of both solvated electrons and chlorophyll a radical anions. Using $\Delta\varepsilon_{470} = 5.05 \cdot 10^3\ M^{-1}cm^{-1}$ for the solvated electron and $\Delta\varepsilon_{470} = 1.0 \cdot 10^4\ M^{-1}cm^{-1}$ for the chlorophyll a radical anion (Sauer et al., 1965; Fujita et al., 1978) the value $k_{18} = (1.5 \pm 0.2) \cdot 10^{10}\ M^{-1}s^{-1}$ is obtained. This represents the mean value of four kinetic curves corresponding to irradiation doses of 3.3 krad, 5.6 krad, 8.5 krad, and 19.5 krad. Similar rate constants for reactions between solvated electrons and other electron scavengers in 2-propanol have been reported (Watson and Roy, 1972). Thus, this rate constant probably reflects a diffusion-limited electron attachment reaction and gives little information about the electron affinity of chlorophyll a.

The further reactions in the system after formation of the chlorophyll a radical anion are probably complex. As indicated by the kinetic curves shown in Fig. 24, at least two products are formed during the slow 400-μs increase in absorption. At 465 nm the optical absorbance has reached the plateau at 385 μs after the electron pulse, but at 550 nm the absorbance is still increasing beyond 385 μs. Free radicals are formed in high yields in alcohols. Apparently no critical examination of 2-propanol has been made, but for ethanol the total free radical yield has been reported to be $G = 8.5\ (100\ eV)^{-1}$ (Freeman, 1974). Assuming this value to be representative of the radical yield in 2-propanol, it is to be expected that products derived from reactions between chlorophyll a, its radical anion, and the free radicals will be formed in high yields. This general picture is very much like the picture in THF discussed above. However, two important differences should be noticed. Firstly, the solvent radical $(CH_3)_2\dot{C}OH$, which probably is the major radical formed, may act as a reducing agent

$$(CH_3)_2\dot{C}OH + Chla \rightarrow (CH_3)_2CO + Chla^{-\bullet} + H^+_{solv}$$

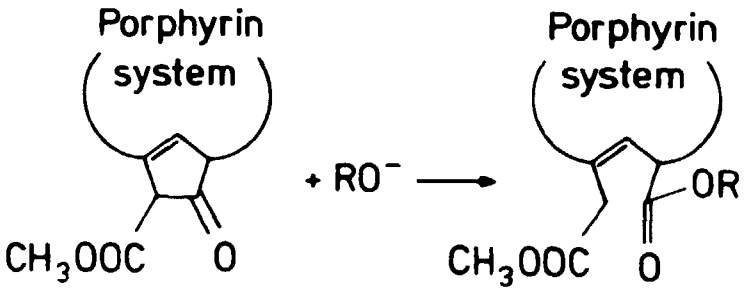
thereby increasing the yield of chlorophyll a radical anion. This reaction should be slow, and it might be responsible for part of the increase in absorbance observed within 400 μs after the electron pulse. Secondly, 2-propanol is a protic medium and consequently protonation of the radical anion is very likely. Protonation of the radical anion was also proposed as a major process in pure THF on the basis of the observed second-order kinetics, but still the kinetic pictures after formation of the radical anion are very different in the two solverts. In THF the optical absorbance decreased in the region 455-480 nm, while in 2-propanol the optical absorbance increased at all wavelengths > 455 nm after the initial formation of the radical anion. This difference might be due to unequal solvation of chlorophyll a and its radical anion in THF and 2-propanol. THF will mainly solvate chlorophyll a by ligating to the central magnesium atom, while 2-propanol will also solvate chlorophyll a strongly in the region of the cyclopentanone ring. Thus, eventual radical attacks on chlorophyll a and its radical anion involving the cyclopentanone ring could be sterically hindered in 2-propanol, thereby inducing a different product distribution in the two solvents.

Finally, the present results should be discussed in relation to a recent report by Neta et al. (1979). These workers studied chlorophyll a in 2-propanol containing 0.1 M of the sodium alcoholate, $(CH_3)_2CHO^-Na^+$. They claimed that the chlorophyll a radical anion was formed under these conditions upon irradiation via reduction by e^-_{solv} and $(CH_3)_2CO^{\cdot-}$.

The reported transient absorption spectrum of the species believed to be the chlorophyll a radical anion is, however, very different from the spectrum reported by Fujita et al. (1979). Unfortunately, spectra obtained in pure 2-propanol were not reported.

In view of the good agreement between the spectra reported here and that obtained by Fujita et al. (1978) it appears justified to assume that the transient species observed by Neta et al. (1979) is not the radical anion of chlorophyll a. Although the identity of the transient of Neta et al. (1979) cannot be estab-

lished on the basis of the available data, it is easy to explain qualitatively why the chlorophyll a radical anion was not formed. As mentioned previously, chlorophyll a is very susceptible to attack by strong bases. The reactions involved may be deprotonation of chlorophyll a at the cyclopentanone ring producing the "phase test intermediate" followed by eventual oxidation of this species. Secondly, strong bases as alcoholates may cleave the cyclopentanone ring of chlorophyll a.



A number of other reactions are possible, depending on the actual conditions. These reactions have all been discussed extensively by Seely (1966) and Svec (1978). Of interest here is the unlikelihood that chlorophyll a would remain unaltered in 2-propanol containing 0.1 M sodium propanolate and that some of the possible degradation products are spectrally similar to chlorophyll a. Hence, the reactions reported by Neta et al. (1979), believed to be reactions of chlorophyll a, must in fact be reactions of some unidentified degradation product, and the transient spectrum must be the radical anion of this degradation product.

REFERENCES

AGALIDIS, I., LUTZ, M. and REISS-HUSSON, F. (1980). Biochim. Biophys. Acta 589, 264-274.

AMAND, B. and BENSASSON, R. (1975). Chem. Phys. Lett. 34, 44-48.

AMESZ, J. (1977). pp. 238-246 in Trebst and Avron (1977).

AMOUYAL, E., BENSASSON, R. and LAND, E.J. (1974). Photochem. Photobiol. 20, 415-422.

AMOUYAL, E. and BENSASSON, R. (1976). Faraday Trans. I 72, 1274-1287.

ANDERSON, A.F.H., CALVIN, M. (1962). Nature (London) 194, 285-286.

BAXENDALE, J.H., BEAUMOND, D. and RODGERS, M.A.J. (1969). Chem. Phys. Lett. 4, 3-4.

BAXENDALE, J.H., BEAUMOND, D. and RODGERS, M.A.J. (1970). Trans. Faraday Soc. 66, 1996-2003.

BAXENDALE, J.H. and FITI, M. (1972). Faraday Trans. II 68, 218-222.

BAXENDALE, J.H. and RODGERS, M.A.J. (1978). Chem. Soc. Rev. 8, 235-263.

BEDDARD, G.S., DAVIDSON, R.S. and TRETHEWEY, K.R. (1977). Nature (London) 267, 373-374.

BENSASSON, R. and LAND, E.J. (1971). Trans. Faraday Soc. 67, 1904-1915.

BENSASSON, R., DAWE, E.A., LONG, D.A. and LAND, E.J. (1977). Faraday Trans I 73, 1319-1325.

BENSASSON, R. and LAND, E.J. (1978). Photochem. Photobiol. Rev. 3, 163-191.

BENSON, S.W. (1960). In the Foundations of Chemical Kinetics, p. 32. McGraw-Hill, New York.

BOWERS, P.G. and PORTER, G. (1967). Proc. R. Soc. London A296, 435-441.

BRETON, J., GEACINTOV, N.E. and SWENBERG, C.E. (1979). Biochim. Biophys. Acta 548, 616-635.

BRIDGE, N.K. and PORTER, G. (1958). Proc. R. Soc. London A244, 259-275; 276-288.

BRIDOUX, M. and DELHAYE, M. (1976). Adv. Infrared Raman Spectrosc. 2, 140-152.

CAREY, P.R. (1978). Quart. Rev. Biophys. 11, 309-370.

CAREY, P.R. and SCHNEIDER, H. (1978). Acc. Chem. Res. 11, 122-128.

CHESSIN, M., LIVINGSTON, R. and TRUSCOTT, T.G. (1966). Trans. Faraday Soc. 62, 1519-1524.

COOPER, R. and THOMAS, J.K. (1968). J. Chem. Phys. 48, 5097-5102.

COTTON, T.M. and VAN DUYNE, R.P. (1978). Biochem. Biophys. Res. Comm. 82, 424-433.

DAINTON, F., MORROW, T., SALMON, G.A. and THOMPSON, G.F. (1972). Proc. R. Soc. Lond. A328, 457-479.

DAINTON, F.S. (1975). Chem. Soc. Rev. 4, 323-362.

DALLINGER, R.F., WOODRUFF, W.H. and RODGERS, M.A.J. (1979). Appl. Spectrosc. 33, 522-523.

DODELET, J.-P. and FREEMAN, G.R. (1975). Can. J. Chem. 53, 1263-1274.

DORFMAN, L.M. (1974). In Techniques of Chemistry, Vol. VI, Part II (Edited by G.G. Hammes), pp. 463-519. Wiley-Interscience, New York.

FAJER, J., BRUNE, D.C., DAVIS, M.S., FORMAN, A. and SPAULDING, L.D. (1975). Proc. Natl. Acad. Sci. USA 72, 4956-4960.

FEHER, G. and OKAMURA, M.Y. (1978). In the Photosynthetic Bacteria (Edited by R.K. Clayton and W.R. Sistrom), pp. 349-386. Plenum Press.

FELTON, R., SHERMAN, G.M. and LINSCHITZ, H. (1964). Nature (London) 203, 637-639.

FLORYAN, E.S. and PAGSBERG, P. (1976). Int. J. Radiat. Phys. Chem. 8, 425-432.

FRANK, H.A., McLEAN, M.B., SAUER, K. (1979). Proc. Natl. Acad. Sci. USA 76, 5124-5128.

FREEMAN, G.R. (1974). NSRDS-NBS 48, National Bureau of Standards, Washington.

FUJITA, I., DAVIS, M.S. and FAJER, J. (1978). J. Am. Chem. Soc. 100, 6280-6282.

GOEDHEER, J.C. (1966), pp. 147-184 in Vernon and Seely (1966). Govindjee (Ed.) (1975). Bioenergetics of Photosynthesis, Academic Press, New York.

GROSSWEINER, L.I. (1978). Photochem. Photobiol. 28, 97-108.

GUPTA, S.K., JONES, E.G., HARRISON, A.G. and MYHER, J.J. (1967). Can. J. Chem. 45, 3107-3117.

HANSEN, K.B., WILBRANDT, R. and PAGSBERG, P. (1979). Rev. Sci. Instrum. 50, 1532-1538.

HARDWICK, T.J. (1966). Actions Chim. Biol. Radiat. 10, 125-185.

HENGLEIN, A. and GRÄTZEL, M. (1977). In Solar Power and Fuels (Edited by J.R. Bolton), pp. 53-76. Academic Press, New York.

HERMANN, H. and SCHENCK, G.O. (1968). Photochem. Photobiol. 8, 255-258.

HESTER, R.E. (1978). Adv. Infrared Raman Spectrosc. 4, 1-45.

HYNNINEN, P.H., WASIELEWSKI, M.R. and KATZ, J.J. (1979). Acta Chem. Scand. B33, 637-648.

JAGENDORF, A.T. (1975), pp. 413-492 in Govindjee (1975).

JANZEN, A.F. and BOLTON, J.R. (1979). J. Am. Chem. Soc. 101, 6342-6348.

JENSEN, N.-H. (1977). Risø-M-1914.

JENSEN, N.-H., WILBRANDT, R., PAGSBERG, P., SILLESEN, A.H. and Hansen, K.B. (1978). J. Photochem. 9, 227-229.

JENSEN, N.-H., WILBRANDT, R. and PAGSBERG, P. (1980). Photochem. Photobiol. (in press).

JOHNSON, B.D. and PETICOLAS, W.L. (1976). Ann. Rev. Phys. Chem. 27, 465-491.

JOU, F.Y. and DORFMAN, L.M. (1973). J. Chem. Phys. 58, 4715-4723.

KAMEN, M.D. (1963). Primary Processes in Photosynthesis. Academic Press, New York.

KAUFMANN, K.J., DUTTON, P.L., NETZEL, T.L., LEIGH, J.S. and RENTZEPIS, P.M. (1975). Science 188, 1301-1304.

KEENE, J.P., KEMP, T.J. and SALMON, G.A. (1965). Proc. R. Soc. London A287, 494-509.

KEMP, D.R. and PORTER, G. (1971). Proc. R. Soc. London, A326, 117-130.

KLIMOV, V.V., KLEVANIK, A.V., SHUVALOV, V.A. and KRASNOVSKY, A.A. (1977). FEBS Lett. 82, 183-186.

KRASNOVSKY, A.A. (1960). Ann. Rev. Plant Physiol. 11, 363-410.

LAMOLA, A.A. (1969). In Energy Transfer and Organic Photochemistry (Edited by P.A. Leermakers and A. Weissberger). Technique of Organic Chemistry. Vol. XIV. pp. 17-132. Wiley-Interscience, New York.

LAFFERTY, J., LAND, E.J. and TRUSCOTT, T.G. (1976). J.C.S. Chem. Comm. 70-71.

LAFFERTY, J., TRUSCOTT, T.G. and LAND, E.J. (1978). Faraday Trans I 74, 2760-2762.

LAND, E.J. (1969). Trans. Faraday Soc. 65, 2815-2822.

LAND, E.J., SYKES, A. and TRUSCOTT, T.G. (1971). Photochem. Photobiol. 13, 311-320.

LENNON, J. and McCARTNEY, P. (1967). On Sgt. Pepper's Lonely Hearts Club Band, EMI Records.

LEVANON, H. and NORRIS, J.R. (1978). Chem. Rev. 78, 185-198.

LINSCHITZ, H. and SARKANEN, K. (1958). J. Am. Chem. Soc. 80, 4826-4832.

LINSCHITZ, H., STEEL, C. and BELL, J.A. (1962). J. Phys. Chem. 66, 2574-2576.

LIVINGSTON, R. (1960).Q. Rev. Chem. Soc. 14, 174-199.

LUTZ, M. (1974). J. Raman Spectrosc. 2, 497-516.

LUTZ, M. (1977). Biochim. Biophys. Acta 460, 408-430.

LUTZ, M., AGALIDIS, I., HERVO, G., COGDELL, R.J. and REISS-HUSSON, F. (1978). Biochim. Biophys. Acta 503, 287-303.

LUTZ, M. and KLEO, J. (1979). Biochim. Biophys. Acta 546, 365-369.

LUTZ, M., BROWN, J.S. and RÉMY, R. (1979), pp. 105-125 in Porter (1979).

MATHIS, P. and KLEO, J. (1973). Photochem. Photobiol. 18, 343-346.

MEISEL, D. and CZAPSKI, G. (1975). J. Phys. Chem. 79, 1503-1509.

NAFISI-MOVAGHAR, J. and WILKINSON, F. (1970). Trans. Faraday Soc. 66, 2257-2267; 2268-2278.

NETA, P., SCHERZ, A. and LEVANON, H. (1979). J. Am. Chem. Soc. 101, 3624-3629.

OLSON, J.M. and HIND, G. (Eds.) (1977). Chlorophyll-proteins, reaction centers and photosynthetic membranes. BNL 50530. Brookhaven National Laboratory, Upton, NY.

PAGSBERG, P., WILBRANDT, R., HANSEN, K.B. and WEISBERG, K.V. (1976). Chem. Phys. Lett. 39, 538-541.

PAGSBERG, P., ERIKSEN, J. and CHRISTENSEN, H.C. (1977). J. Phys. Chem. 83, 582-590.

PARKER, C.A. and JOYCE, T.A. (1967). Photochem. Photobiol. 6, 395-406.

PORTER, G. and WRIGHT, M.R. (1959). Disc. Faraday Soc. 27, 18-27.

PORTER, G. and WILKINSON, F. (1961). Proc. R. Soc. London A264, 1-18.

PORTER, G. and WEST, M.A. (1974). In Techniques of Chemistry, Vol. VI, Part II (Edited by G.G. Hammes), pp. 367-462. Wiley-Interscience, New York.

PORTER, G. (1978). Proc. R. Soc. Lond. A362, 281-303.

PORTER, G. (chairman) (1979). Chlorophyll Organization and Energy Transfer in Photosynthesis. Ciba Foundation Symposium 61 (new series). Excerpta Medica, Amsterdam.

ROBINSON, A.J. and RODGERS, M.A.J. (1975). J.C.S. Faraday Trans I 71, 378-388.

ROBINSON, G.W. (1963). Proc. Natl. Acad. Sci. USA 49, 521-529.

ROCKLEY, M.G., WINDSOR, M.W., COGDELL, R.J. and PARSON, W.W. (1975). Proc. Natl. Acad. Sci. USA 72, 2251-2255.

SALMON, G.A., SEDDON, W.A. and FLETCHER, J.W. (1974). Can. J. Chem. 52, 3259-3268.

SALMON, G.A. (1976). Int. J. Radiat. Phys. Chem. 8, 13-32.

SANADI, D.R. and VERNON, L.P. (Eds.) (1978a). Curr. Top. Bioenergetics, Vol. 7. Academic Press, New York.

SANADI, D.R. and VERNON, L.P. (Eds.) (1978b). Curr. Top. Bioenergetics, Vol. 8, Academic Press, New York.

SAUER, K., SMITH, J.R.L. and SCHULTZ, A.J. (1966). J. Am. Chem. Soc. 88, 2681-2688.

SAUER, K. (1978). Acc. Chem. Res. 11, 257-264.

SAUER, K. (1979). Ann. Rev. Phys. Chem. 30, 155-178.

SAUER, M.C., ARAI, S. and DORFMAN, L.M. (1965). J. Chem. Phys. 42, 708-712.

SCHEER, H. and KATZ, J.J. (1974). Proc. Natl. Acad. Sci. USA 71, 1626-1629.

SCHEERER, R. and GRÄTZEL, M. (1976). Ber. Bunsenges. Phys. Chem. 80, 979-982.

SCHEERER, R. and GRÄTZEL, M. (1977). J. Am. Chem. Soc. 99, 865-871.

SCHINDEWOLF, U. (1978). Angew. Chem. 90, 939-954.

SCHMIDT, W.F. and ALLEN, A.O. (1968). J. Phys. Chem. 72, 3730-3736.

SCHMIDT, W.F. and ALLEN, A.O. (1970). J. Chem. Phys. 52, 2345-2351.

SEARLE, G.F.W. and WESSELS, J.S.C. (1978). Biochim. Biophys. Acta 504, 84-99.

SEELY, G.R. and JENSEN, R.G. (1965). Spectrochim. Acta 21, 1835-1845.

SEELY, G.R. (1966). pp. 67-109 in Vernon and Seely (1966).

SEELY, G.R. (1978). pp. 3-37 in Sanadi and Vernon (1978a).

SHIPMAN, L.L., COTTON, T.M., NORRIS, J.R. and KATZ, J.J. (1976). J. Am. Chem. Soc. 98, 8222-8230.

SHUVALOV, V.A., DOLAN, E. and KE, B. (1979). Proc. Natl. Acad. Sci. USA 76, 770-773.

SINGH, A. (1972). Radiation Res. Rev. 4, 1-69.

SPINKS, J.W.T. and WOODS, R.J. (1976). An Introduction to Radiation Chemistry. Wiley-Interscience, New York.

SPIRO, T.G. and STEIN, P. (1977). Ann. Rev. Phys. Chem. 28, 501-521.

STRAIN, H.H. and SVEC, W.A. (1966). pp. 21-66 in Vernon and Seely (1966).

SVEC, W.A. (1978). In the Porphyrins, Vol. V (Edited by D. Dolphin). pp. 341-399. Academic Press, New York.

SYMONS, M.C.R. (1976). Chem. Soc. Rev. 5, 337-358.

TFIBEL, F. and LINDQVIST, L. (1975). Chem. Phys. 10, 471-478.

THOMAS, J.K. (1976). Int. J. Radiat. Phys. Chem. 8, 1-12.

THORNBER, J.P., ALBERTE, R.S., HUNTER, F.A., SHIOZOWA, J.A. and KAN, K.-S. (1977). pp.132-148 in Olsen and Hind (1977).

THRASH, R.J., FANG, H.L.B. and LEROI, G.E. (1977). J. Chem. Phys. 67, 5930-5933.

THRASH, R.J., FANG, H.L.B. and LEROI, G.E. (1979). Photochem. Photobiol. 29, 1049-1050.

TIEN, H.T. (1977). pp. 105-131 in Olsen and Hind (1977).

TREBST, A. and AVRON, M. (Eds.) (1977). Photosynthesis I. Encyclopedia of Plant Physiology, New Series, Vol. 5. Springer-Verlag, Berlin.

TRUSCOTT, T.G., LAND, E.J. and SYKES, A. (1973). Photochem. Photobiol. 17, 43-51.

VERNON, L.P. and SEELY, G.R. (Eds.) (1966). The chlorophylls. Academic Press, New York.

VERNON, L.P., SHAW, E.R., OGAWA, T. and RAVEED, D. (1971). Photochem. Photobiol. 14, 343-357.

WATSON, E. and ROY, S. (1972). NSRDS-NBS 42. National Bureau of Standards, Washington.

WELLER, A. (1954). J.Am. Chem. Soc. 76, 5819-5821.

WESTON, R.E. and SCHWARZ, H.A. (1972). Chemical Kinetics, pp. 151-187. Prentice-Hall, Englewood Cliffs.

WILBRANDT, R., PAGSBERG, P., HANSEN, K.B. and WEISBERG, K.V. (1975). Chem. Phys. Lett. 36, 76-78.

WILKINSON, F., SEDDON, G.M. and TICKLE, K. (1968). Ber. Bunsenges. Phys. Chem. 72, 315-321.

WITT, H.T. (1971). Quart. Rev. Biophys. 4, 365-477.

WITT, H.T. (1975). pp. 493-554 in Govindjee (1975).

APPENDIX

TIME-RESOLVED RESONANCE RAMAN SPECTROSCOPY: THE EXCITED TRIPLET STATE OF ALL-trans-β-CAROTENE

Niels-Henrik Jensen, Robert Wilbrandt, Palle B. Pagsberg, A.H. Sillesen and K.B. Hansen

Chemistry Department
Risø National Laboratory
DK-4000 Roskilde
Denmark

Abstract. The lowest excited triplet state of all-trans-β-carotene produced by pulse radiolysis has been studied by time-resolved resonance Raman spectroscopy. Six transient Raman bands at 965 cm^{-1}, 1009 cm^{-1}, 1125 cm^{-1}, 1188 cm^{-1}, 1236 cm^{-1} and 1496 cm^{-1} were observed. The assignment of these transient bands to the triplet state of β-carotene is based on measurements of the dependence of the intensity upon irradiation dose and time. The Raman intensity was found to have the same dose and time dependence as the transient optical absorbance. The spectrum is discussed in detail and it is concluded that the C = C double bond order is decreased in the triplet state and that the molecule may be substantially twisted, presumably at the 15,15' bond, in the triplet state. The downward shift of the strong band in the C-C stretching region to 1125 cm^{-1}, however, still remains unexplained.

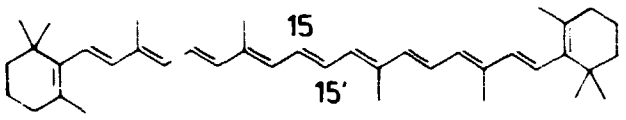
(Manuscript accepted for publication in Journal of the American Chemical Society)

INTRODUCTION

The study of excited states of conjugated polyenes such as carotenes is of considerable interest in the understanding of photosynthesis. Previous studies of the lowest excited triplet state of all-trans-β-carotene in vitro include flash photolysis[1,2] and pulse radiolysis[3-5] experiments. All of these are based on the observation of a very strong transient optical absorption in the 510-530 nm region, which is due to the lowest triplet-triplet transition in β-carotene.

β-carotene and related polyenes are also known to exhibit extremely strong enhancement in resonance Raman scattering. A large number of workers[6-15] have reported on different aspects of the resonance Raman spectrum of the ground-state of β-carotene.

A few years ago the technique of resonance Raman spectroscopy has been extended by us to the study of short-lived radicals[16,17] and excited triplet states[18] and other groups[19-23] have reported on similar work. Recently Woodruff and coworkers[24] published the first time-resolved resonance Raman spectrum of the lowest triplet state of all-trans-β-carotene using pulse radiolysis. These authors detected three vibrational bands at 1495 cm^{-1}, 1126 cm^{-1} and 1014 cm^{-1}, which were correlated to the ground-state in-phase double bond C = C stretching mode at 1521 cm^{-1}, the C-C in-plane single bond stretching mode at 1157 cm^{-1} and a C-H in-plane bending mode at 1005 cm^{-1} with the respective shifts when going to the triplet state. We report here a study under similar conditions essentially confirming the results of Woodruff and coworkers, but additionally finding three vibrational bands of the transient excited state at 965 cm^{-1}, 1188 cm^{-1} and 1236 cm^{-1} and giving details with respect to the dependence of the vibrational spectra on time and on the delivered irradiation dose.



ALL - trans β - CAROTENE

EXPERIMENTAL

All-trans-β-carotene was supplied from Sigma and used without further purification. Naphthalene (scintillation grade) was obtained from Merck. Benzene (Merck p.a.) was passed through a column packed with neutral alumina (Woelm) before use. 10^{-4} M solutions of β-carotene in benzene containing 10^{-2} M of naphthalene as sensitizer in order to populate the polyene triplet state efficiently were flushed ½ hour with Ar before using. The electron irradiation facilities and the electro-optical multichannel spectrometer for the detection of Raman scattered light are described in detail elsewhere[25]. Only the essential features shall be given here. Solutions were irradiated with a pulse of 2 MeV electrons of 30 ns duration and changes in optical absorption were detected in the usual way by analysing the light from a Xe-lamp transmitted through the reaction cell. Resonance Raman spectra were excited by a pulse of 600 ns duration from a tunable dye laser with variable time delay with respect to the electron pulse. The transient resonance Raman spectra of triplet β-carotene reported below were all excited at 531.1 nm. The scattered light was detected by menas of an optical multichannel spectrometer gated for 1 μs in order to discriminate against Cerenkov light. The data were processed by a computer. The spectral resolution of the system was limited by the laser bandwidth to 20 cm^{-1}, in order to obtain sufficient laser pulse energy. The main differences compared to the work of Dallinger et al. are 1) the higher irradiation dose of ca. 68 krad in our experiments which allowed the generation of excited states at correspondingly higher concentrations, 2) the

longer laser pulse duration, and 3) the fact that our spectra are recorded with single pulses, changing the solution after each pulse.

RESULTS AND DISCUSSION

The spectral and kinetic characteristics of the chemical system, 10^{-4} M β-carotene in benzene containing 10^{-2} M naphthalene, were investigated in detail by optical absorption spectroscopy. These measurements, which will be reported elsewhere[26], gave several results of importance for the present study.

The absorption spectrum obtained with an irradiation dose of 1.7 krad had λ_{max} = 530 nm which compares well with the results of Mathis and Kleo[2] obtained by chlorophyll a sensitized flash photolysis of β-carotene in benzene. However, this value differs from the value of 515 nm reported by Dallinger et al.[24]. Presumably they used the value obtained by Land et al.[3] with n-hexane as solvent.

In estimating the radiation chemical yield of triplet β-carotene we first calibrated the relative doses by measuring the optical densities of triplet naphthalene obtained by irradiation of 10^{-2} M naphthalene in benzene. By using $G \cdot \Delta\varepsilon_{max} = 3.58 \cdot 10^4$ (100 eV)$^{-1}$ M^{-1}cm^{-1}[27] for triplet naphthalene the effective doses could be calculated. From our measurements on β-carotene we obtained $G \cdot \Delta\varepsilon_{530} = (2.75 \pm 0.25) \cdot 10^5$ (100 eV)$^{-1}$ M^{-1}cm^{-1} for the production of triplet β-carotene in benzene containing 10^{-2} M naphthalene and 10^{-4} M β-carotene. Using $\Delta\varepsilon_{max} = 2.38 \cdot 10^5$ M^{-1}cm^{-1}[4] we get G = 1.15 (100 eV)$^{-1}$ for the effective yield of triplet β-carotene. As $\Delta\varepsilon_{max} = 1.32 \cdot 10^4$ M^{-1}cm^{-1} for triplet naphthalene in benzene[28] this G-value indicates that ca. 42% of the triplet naphthalene molecules were quenched by β-carotene.

The G-value for formation of triplet β-carotene was constant up to a dose of at least 25 krad as plots of maximum absorbance versus dose were linear[26]. The G-value probably decreases at higher irradiation doses, but we estimate that approximately 50% of the β-carotene molecules were excited to the triplet state at the maximum dose used for resonance Raman experiments.

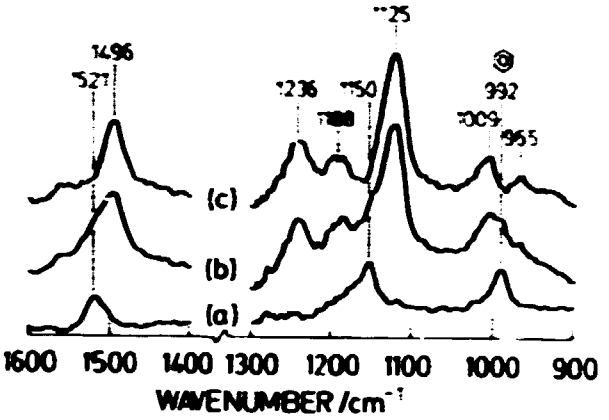


Fig. 1. Resonance Raman spectra from 10^{-4} M all-trans-β-carotene in benzene containing 10^{-2} M naphthalene.
a) Background spectrum without electron irradiation.
b) Spectrum recorded 1 μs after electron irradiation.
c) Difference spectrum b) - a).
Irradiation dose = 68 krad.
Laser excitation wavelength = 531.1 nm.
Spectral segments recorded with single pulses.

Figure 1b shows the resonance Raman spectrum obtained with an irradiation dose of ca. 68 krad. The delay of the laser pulse with respect to the electron pulse was adjusted so the sample was probed at a time where the triplet concentration was near its maximum. Figure 1a shows the background spectrum without electron irradiation and Fig. 1c if the difference spectrum 1b - 1a showing the bands of the transient only. The vibrational frequencies are tabulated in Table I along with data on ground-state β-carotene and the results of Dallinger et al.[24]. Before proceeding with a discussion of these experimental observations we present evidence for the assignment of the transient resonance Raman bands to the excited triplet state of β-carotene.

Table I. Vibrational frequencies/cm^{-1}

Ground state			Triplet excited state		
Rimai et al.[a] cw-excit. n-hexane 488 nm	This work cw-excit. Benzene 514.5 nm	Dallinger et al.[b] Pulsed Benzene 531.8 nm	This work Pulsed Benzene 531.1 nm	Dallinger et al.[b] Pulsed Benzene 531.8 nm	This work Pulsed Benzene 531.1 nm
961 vw	-	-	-	-	965±13 w
1006 w	1005 w	1003	-	1014	1009±3 m
1158 vs	1157 vs	1157	1150	1126	1125±2 vs
1193 w	1190 w	-	-	-	1188±3 m
1215 vw	1213 vw	-	-	-	1236±3 s
1527 vs	1520 vs	1521	1521	1521	1496±2 vs

a) Reference 7
b) Reference 24

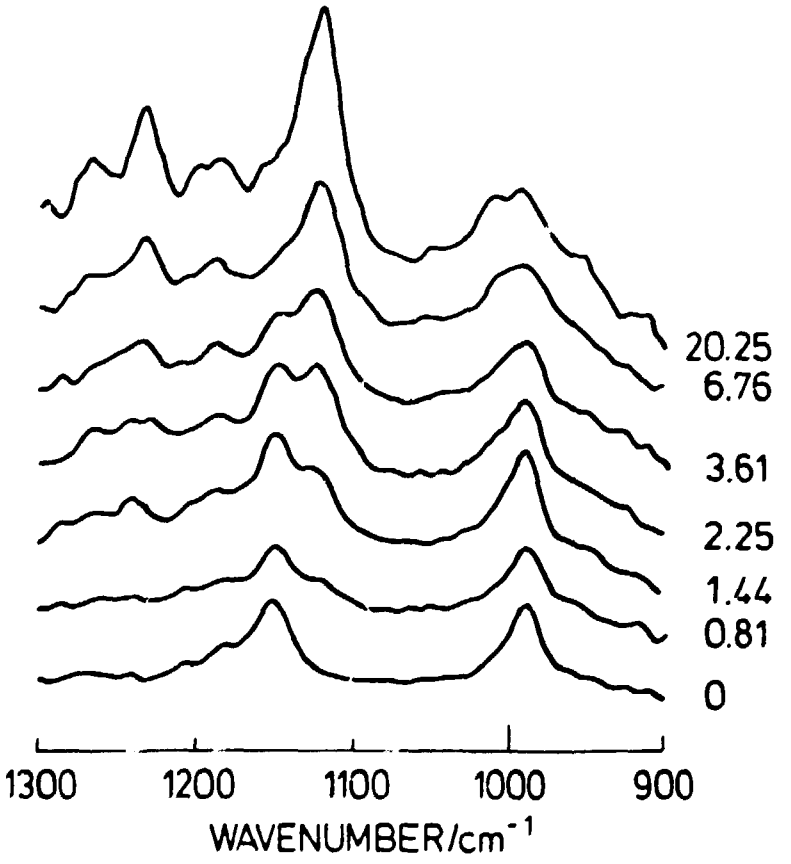


Fig. 2. Resonance Raman spectra recorded after electron irradiation of 10^{-4} M all-*trans*-β-carotene in benzene containing 10^{-2} M naphthalene with the relative doses indicated. Laser excitation wavelength = 531.1 nm. All spectra were recorded 1.7 μs after the electron pulse except the spectrum at highest dose, which was recorded 1 μs after the electron pulse.

Figure 2 shows a series of resonance Raman spectra obtained at various irradiation doses. The doses varied between 2.8 and 68 krad. For comparison a background spectrum without irradiation, but under otherwise identical conditions, is shown in the lower trace. It can be seen that the intensity of the transient Raman

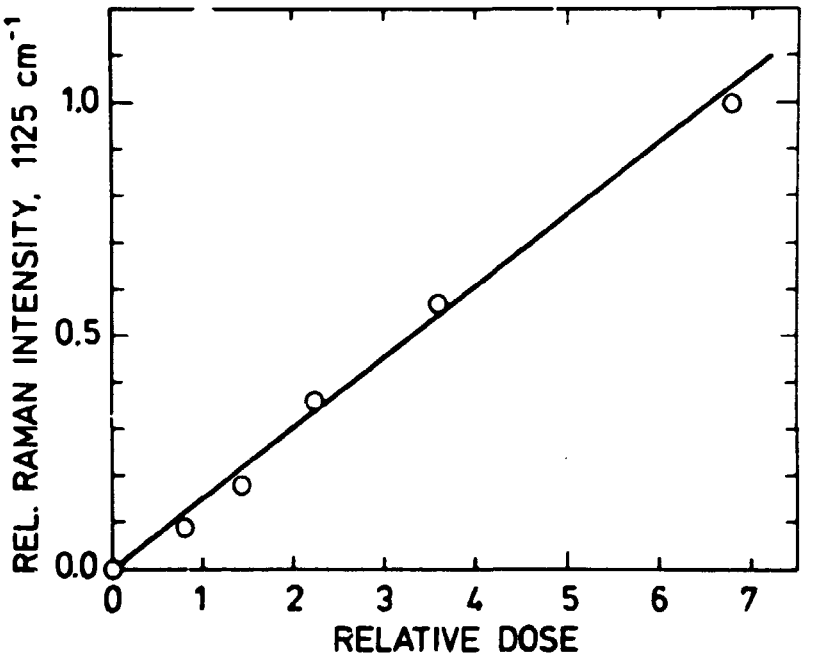


Fig. 3. Raman intensity at 1125 cm^{-1} versus relative irradiation dose. See text for discussion.

bands increases with increasing dose. Figure 3 shows the relative Raman intensity at 1125 cm^{-1} as a function of the relative irradiation dose. The points were obtained by measuring the 1125 cm^{-1} intensity relative to the intensity of the benzene band at 992 cm^{-1} in the spectra shown in Fig. 2, neglecting the difference in absorbance due to different wavenumbers[29]. Thus the transient optical absorbance[26] and the transient Raman intensity have qualitatively the same dose dependence, indicating a common origin of the two observable quantities. The transient band at 1125 cm^{-1} is still detectable at a relative dose of 0.81 (2.8 krad) corresponding to a triplet concentration of ca. $3.3 \cdot 10^{-6}$ M.

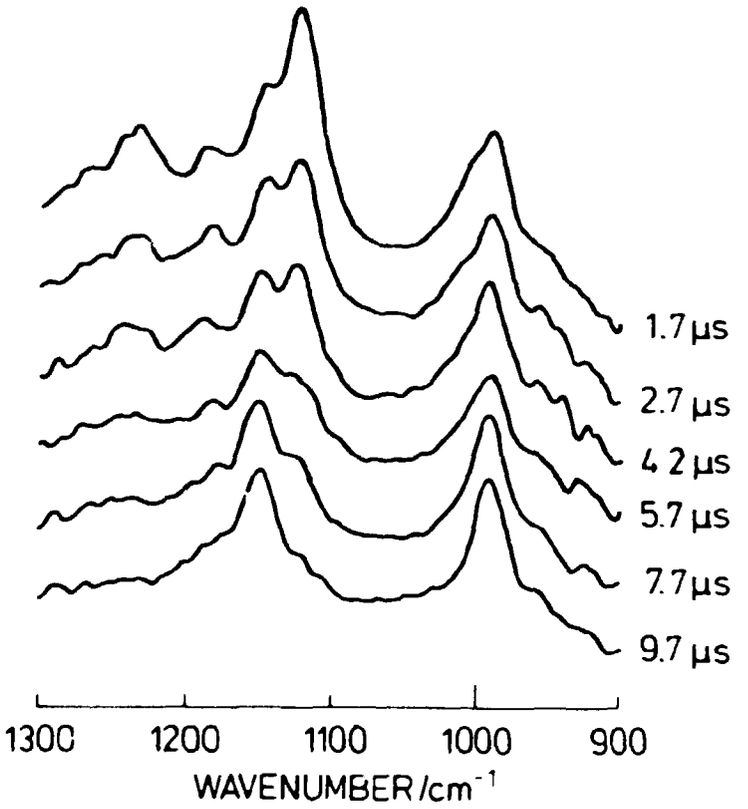


Fig. 4. Resonance Raman spectra recorded at various delays of laser pulse with respect to electron pulse. Irradiation dose was constant = 17.6 krad. Solution: 10^{-4} M all-trans-β-carotene in benzene containing 10^{-2} M naphthalene. Laser excitation wavelength = 531.1 nm.

Figure 4 shows a series of spectra obtained at various delays after the electron pulse, but at a constant relative irradiation dose of 5.29. This dose corresponds to 17.6 krad and lies within the dose range where a linear relation between Raman intensity and irradiation dose was found (Fig. 3). The spectrum obtained at 1.7 µs delay corresponds to maximum transient concentration. From these spectra an effective halflife of 2.5±0.5 µs is obtained. This value is in good agreement with the value 2.3 µs obtained from the transient optical absorbance measurement at identical irradiation dose as shown in Fig. 5. As seen in Fig. 4, all transient bands show qualitatively the same time dependence. On the basis of the dose and time dependence of the transient Raman bands we are confident that these bands originate

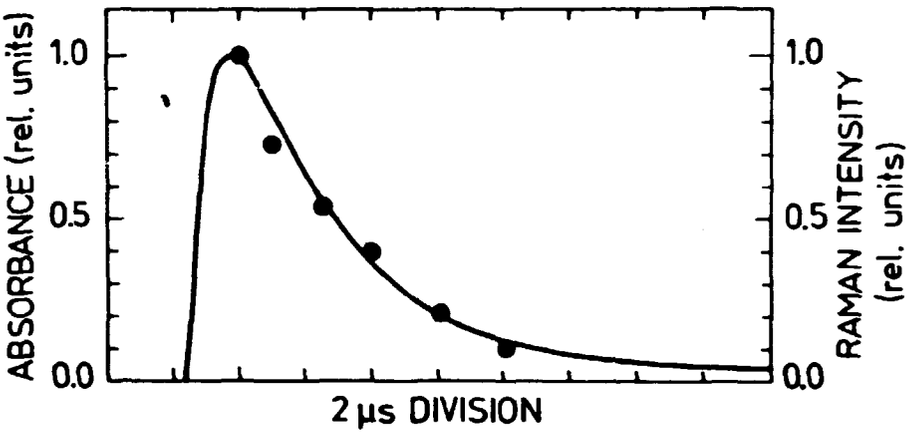


Fig. 5. Optical absorbance (full line) and Raman intensity at 1125 cm^{-1} (points) versus time. Conditions as in Fig. 4.

from the lowest excited triplet state of β-carotene. No indication of photoisomerization products from the ground state appeared.

Rimai et al.[8] have presented a vibrational analysis of the polyenes including β-carotene. Their important conclusions were that the bands at ca. 1500 cm^{-1} are adequately described as C = C stretching modes, the frequency being sensitive to the double bond character of the formal C = C bonds. The bands in the 1100-1300 cm^{-1} region contain large contributions from C-C stretching and C-C-H in-plane deformations as well as some C = C stretching. However, the bands in this region cannot be described in terms of a single type of bond. For β-carotene the band 1005 cm^{-1} is most likely due to $C-CH_3$ stretching as this band is present in all natural polyenes containing the isoprenoid elements. Finally, the band at 961 cm^{-1} can be described as in-plane angular deformation of C-C-C and C-C-H.

With these qualitative assignments in mind we are now able to discuss the spectral features observed for the lowest excited triplet state. When correlating the triplet Raman bands with the bands of the parent ground-state molecule, frequencies as well as intensities should be taken into account. The triplet bands at 965 cm^{-1} and 1009 cm^{-1} are most likely due to the same

modes as the parent bands at 961 cm^{-1} and 1005 cm^{-1} because of their frequencies and relative intensities. This correlation is supported by the descriptive assignments given above for the parent bands. Firstly, the frequencies of C-C-C/C-C-H bending modes have previously been predicted to be nearly independent of partial bond order changes[30], and recent experimental resonance Raman studies of radical ions have confirmed these predictions[31]. Secondly, the $C-CH_3$ bonds are largely unconjugated with the π-electrons of the alternating double bonds in the polyene chain. Thus, neither the C-C-C/C-C-H angular deformation modes nor the $C-Ch_3$ stretching modes are expected to be influenced by the electronic excitation.

The bands at 1125 cm^{-1}, 1188 cm^{-1} and 1236 cm^{-1} show an intensity pattern not found in the ground-state spectrum (see Table I). Only the band at 1125 cm^{-1} seems to be correlated with the ground-state band at 1157 cm^{-1} indicating a shift of 32 cm^{-1} to lower frequency. This frequency shift will be discussed later. The bands at 1188 cm^{-1} and 1236 cm^{-1} have intensities relative to the 1125 cm^{-1} band which do not justify direct correlation with the ground-state bands at 1190 cm^{-1}. In particular the transient band at 1236 cm^{-1} is much stronger compared to the bands at 1125 cm^{-1} and 1188 cm^{-1} than the ground-state band at 1213 cm^{-1} compared to the bands at 1157 cm^{-1} and 1190 cm^{-1}. Furthermore, it should be noted that the high relative intensities of the 1188 cm^{-1}/1236 cm^{-1} bands probably are not due to specific enhancement effects, as the intensity pattern of the spectra did not change by using laser excitation at longer wavelengths[32]. Thus, the transient bands in the 1100-1300 cm^{-1} region most likely reflect a configurational change of all-<u>trans</u>-β-carotene in the excited triplet state as compared with the ground state.

Lutz et al.[33] have reported on the low-temperature resonance Raman spectra of all-<u>trans</u>-β-carotene and 15,15'-cis-β-carotene. The most striking difference in the spectra of the two isomers is the appearance of a pair of strong bands at 1245 cm^{-1}/1237 cm^{-1}, and an intensity enhancement of the bands at 1194 cm^{-1} and 955 cm^{-1} in the spectra of the cis-isomer. The strong band at 1160 cm^{-1} remains unshifted while the band at 1530 cm^{-1}

shifts to 1540 cm^{-1} in the cis-isomer. Rimai et al.[34,35] have investigated the Raman spectra of a series of isomers of retinal. They found that the most prominent changes in the spectra occurred in the 1100 cm^{-1} - 1400 cm^{-1} region. In particular the various cis-isomers are characterized by specific new bands in this spectral region as compared to the all-trans isomer.

Taking these observations into account we believe that the observed changes in the Raman spectra on going from the parent ground-state compound to the lowest excited triplet state indicate substantial C = C bond twisting in the equilibrium conformation of the triplet state. In particular the new feature at 1236 cm^{-1} in the triplet spectrum suggests that the bond twisting of the triplet-excited β-carotene is localized at the 15,15' double bond.

The interpretation of the triplet Raman spectrum suggested here is qualitatively supported by the similarity between the triplet-triplet absorption spectra of all-trans-β-carotene and 15,15'-cis-β-carotene[4,5] and by theoretical predictions based on molecular orbital calculations[36,37]. As far as the present discussion is concerned these calculations predicted double bond twisting of the polyenes in their excited states. Twisting of the inner double bonds was preferred energetically as compared with twisting of outer double bonds.

The remaining band at 1496 cm^{-1} in the triplet spectrum can be correlated with the ground-state band at 1520 cm^{-1} resulting in a shift of 24 cm^{-1}. As the ground-state band at 1520 cm^{-1} probably is due to in-phase C = C stretching vibrations the observed shift upon excitation can be explained qualitatively by the decreased π-bond order in the excited state which have been predicted[36,37].

Lutz et al.[33] reported that the strong C = C band of all-trans-β-carotene at 1530 cm^{-1} in their spectra shifted to 1540 cm^{-1} in the spectrum of 15,15'-cis-β-carotene. Assuming that our interpretation of the triplet spectrum of all-trans-β-carotene given above is adequate we may notice that two opposing

effects are operating. One is the electronic effect caused by the electronic excitation and the other the conformational effect due to the change in equilibrium geometry. Thus the shift due to the electronic excitation alone should be greater than 24 cm^{-1}.

Having discussed the frequency shift of the 1520 cm^{-1} ground-state bands in terms of the decreased π-bond order of the excited state, the 32 cm^{-1} downward shift of the 1157 cm^{-1} ground-state band remains to be explained. Although the 1157 cm^{-1} mode contains a minor contribution from C = C-stretching according to Rimai et al.[8] the shift can hardly be explained by the theoretically predicted decrease in π-bond order excitation as this decrease should be accompanied by a bond order increase in the single bond regions. Twisting of the 15,15'-bond in the excited state cannot explain the shift either, as Lutz et al.[33] reported the strong band at 1160 cm^{-1} in both isomers. On the basis of their resonance Raman spectrum of the triplet β-carotene Dallinger et al.[24] proposed that the 1005 cm^{-1} band might be due to the C-C-stretching mode, as they observed a minor upwards frequency shift for this band. Our measurements do not support this assignment as the shift from 1005 cm^{-1} to 1009 cm^{-1} is insignificant; thus they agree with the assignment of this band to the $C-CH_3$ stretching mode. In conclusion we must leave the question open and note, as Dallinger et al. did, that these resonance Raman data may indicate that the force constants in the C-C stretching region are not changing in the predicted manner. Studies on related structures might help in resolving this problem.

CONCLUSIONS

The resonance Raman spectrum of the lowest excited triplet state of all-<u>trans</u>-β-carotene has been obtained and the assignment of the transient bands supported by investigating the

dose and time dependence of the Raman intensity. The frequency and intensity changes observed on going from the ground-state molecule to the triplet-excited molecule suggest that the all-trans-β-carotene in its lowest excited triplet state is twisted, presumably about the 15,15'-bond. The shift observed for the ground-state band at 1157 cm^{-1}, however, cannot be qualitatively explained in terms of the suggested geometry changes.

ACKNOWLEDGEMENTS

The authors are grateful to Paul-Anker Lund (University of Copenhagen) for recording the cw-excited resonance Raman spectrum of all-trans-β-carotene and to Jytte Eriksen, Anne Nielsen and Preben Genske for technical assistance.

REPERENCES

1) M. CHESSIN, R. LIVINGSTON and T.G. TRUSCOTT. Trans. Faraday Soc. 62, 1519 (1966).
2) P. MATHIS and J. KLEO. Photochem. Photobiol. 18, 343 (1973).
3) E.J. LAND, A. SYKES and T.G. TRUSCOTT. Photochem. Photobiol. 13, 311 (1971).
4) R. BENSASSON, E.A. DAWE, D.A. LONG and E.J. LAND. Faraday Trans I 73, 1319 (1977).
5) T.G. TRUSCOTT, E.J. LAND and A. SYKES. Photochem. Photobiol. 17, 43 (1973).
6) J. BEHRINGER and J. BRANDMULLER. Ann. Phys. 4, 234 (1959).
7) L. RIMAI, R.G. KILPONEN and D. GILL. J. Am. Chem. Soc. 92, 3824 (1970).

8) L. RIMAI, M.E. HEYDE and D. GILL. J. Am. Chem. Soc. 95, 4493 (1973).

9) M.C. HUTLEY and D.J. JACOBS. Chem. Phys. Lett. 6, 269 (1970).

10) R.J. THRASH, H.L.B. FANG and G.E. LEROI. J. Chem. Phys. 67, 5930 (1977).

11) F. INAGAKI, M. TASUMI and T. MIYAZAWA. J. Mol. Spectrosc. 50, 286 (1974).

12) F. INAGAKI, M. TASUMI and T. MIYAZAWA. J. Raman Spectrosc. 3, 335 (1975).

13) S. SUFRA, G. DELLEPIANE, G. MASETTI and G. ZERBI. J. Raman Spectrosc. 6, 267 (1977).

14) C. TRIC. J. Chem. Phys. 51, 4778 (1969).

15) A. WARSHEL and P. DAUBER. J. Chem. Phys. 66, 5477 (1977).

16) R. WILBRANDT, P. PAGSBERG, K.B. HANSEN and K.V. WEISBERG. Chem. Phys. Lett. 36, 76 (1975). Ibid. 39, 538 (1976).

17) R. WILBRANDT, N.H. JENSEN, P. PAGSBERG, A.H. SILLESEN, K.B. HANSEN and R.E. HESTER. Chem. Phys. Lett. 60, 315 (1979).
N.H. JENSEN, R. WILBRANDT, P. PAGSBERG, E. ERNSTBRUNNER and R.E. HESTER. J. Chem. Phys. 71, 3326 (1979).

18) R. WILBRANDT, N.H. JENSEN, P. PAGSBERG, A.H. SILLESEN and K.B. HANSEN. Nature (London) 276, 167 (1978).

19) R.E. HESTER. Adv. Infrared Raman Spectrosc. 4, Chapter 1 (1978).

20) A. CAMPION, M.A. El-SAYED and J. TERNER. Biophys. J., 20, 369 (1977).

21) W.H. WOODRUFF and S. FARQUHARSON. In: "New Applications of Lasers in Chemistry", G.M. Hieftje, Ed., American Chemical Society, Washington, D.C. (1978).

22) R.B. SRIVASTARA, M.W. SCHUYLER, L.R. DOSSER, F.J. PURCELL and G.H. ATKINSON. Chem. Phys. Lett. 56, 595 (1978).

23) M. BRIDOUX. In: "Proceedings of the 6th International Conference on Raman Spectroscopy", E.D. Schmid, R.S. Krishnan, W. Kiefer and H.W. Schrötter, Eds. (Heyden, London, 1978) p. 381.

24) R.F. DALLINGER, J.J. GUANCI, Jr., W.H. WOODRUFF and M.A.J. RODGERS. J. Am. Chem. Soc. 101, 1355 (1979).

25) K.B. HANSEN, R. WILBRANDT and P. PAGSBERG. Rev. Sci. Instrum. 50, 1532 (1979).

26) a) N.-H. JENSEN, R. WILBRANDT and P. PAGSBERG. Photochem. Photobiol. (in press).
b) N.-H. JENSEN. Risø Report No. 415 (in press).

27) F. DAINTON, T. MORROW, G.A. SALMON and G.F. THOMPSON. Proc. Roy. Soc. London, A328, 457 (1972).

28) R. BENSASSON and E.J. LAND. Trans. Faraday Soc. 67, 1904 (1971).

29) Different transient Raman bands will generally be attenuated differently when the concentration of the transient species (i.e. the transient absorbance) is changed. In the present case, however, the differences in transient absorbance at different nearby wavenumbers can be neglected as the bands at 1125 cm^{-1} and 992 cm^{-1} only spans ca. 4 nm around 563 nm on the wavelength scale. This wavelength is at the red tail of the transient triplet-triplet absorption where the transient absorbance is varying slowly with wavelength[26].

30) H.E. O'NEAL and S.W. BENSON. J. Phys. Chem. 71, 2903 (1967).

31) a) D.L. JEANMAIRE and R.P. VAN DUYNE. J. Am. Chem. Soc. 98, 4029 (1976).
b) E. ERNSTBRUNNER, R.B. GIRLING, W.E.L. GROSSMAN and R.E. HESTER. J. Chem. Soc. Perkin Trans. II 177 (1978).

32) In these experiments the transient Raman spectra of triplet β-carotene were excited at 531.1 nm, 533.1 nm, 535.1 nm and 537.4 nm.

33) M. LUTZ, I. AGALIDIS, G. HERVO, R.J. COGDELL and F. REISS-HUSSON. Biochim. Biophys. Acta 503, 287 (1978).

34) L. RIMAI, D. GILL and J.L. PARSONS. J. Am. Chem. Soc. 93, 1353 (1971).

35) D. GILL, M.E. HEYDE and L. RIMAI. J. Am. Chem. Soc. 93, 6288 (1971).

36) N.C. BAIRD and R.M. WEST. J. Am. Chem. Soc. 93, 4427 (1971).

37) A. WARSHEL and M. KARPLUS. J. Am. Chem. Soc. 96, 5677 (1974).

E R R A T A

Abstract page 2, line 15 and 16: band should read bond

Page 79, line 19: menas should read means

- 82, Table I: see new table
- 86, line 11: $C\text{-}Ch_3$ should read $C\text{-}CH_3$
- 86, line 20: should read 1190 cm^{-1} and 1213 cm^{-1}.
- 58, bottom: should read ..... described reactions no discussion of these experiments will be given here.

Table I. Vibrational frequencies/cm^{-1}

Ground state				Triplet excited state	
Rimai et al.[a] cw-excit. n-hexane 488 nm	This work cw-excit. Benzene 514.5 nm	Dallinger et al.[b] Pulsed Benzene 531.8 nm	This work Pulsed Benzene 531.1 nm	Dallinger et al.[b] Pulsed Benzene 531.8 nm	This work Pulsed Benzene 531.1 nm
961 vw	-	-	-	-	965±13 w
1006 w	1005 w	1003	-	1014	1009±3 m
1158 vs	1157 vs	1157	1150	1126	1125±2 vs
1193 w	1190 w	-	-	-	1188±3 m
1215 vw	1213 vw	-	-	-	1236±3 s
1527 vs	1520 vs	1521	1521	1495	1496±2 vs

a) Reference 7

b) Reference 24

Sales distributors:
Jul. Gjellerup, Sølvgade 87,
DK-1307 Copenhagen K, Denmark

Available on exchange from:
Risø Library, Risø National Laboratory,
P. O. Box 49, DK-4000 Roskilde, Denmark

ISBN 87-550-0710-
ISSN 0106-2840